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PREFACE

The term production disease includes a number of metabolic disorders of increasing importance in agriculture. During the first conference of this kind which was held at Champaign/Urbana, U.S.A. in 1968, the only topic for consideration was milk fever or parturient hypocalcaemia[1].
In the second conference the production disease was placed in a much wider context. The three aspects, input, output and throughput were the themes of the second International Conference on Production Disease, held in 1972 in Great Britain[2].

In the present program you will find back almost all the topics which have been discussed in the previous conferences indicating that the production problems around parturition in the highly producing milking cow are not yet solved although considerable progress was achieved in the insight of metabolic processus directly related with these diseases. It is, however, very important that the advanced knowledge has led to the development of measures in livestock management, in particular in the composition of the ration which contribute nowadays for a considerable part in the prevention of the original metabolic diseases in ruminants,like milk fever, grass-tetany and ketosis.
In breeding fast growing and fertile pigs with a favourable feed conversion, short fattening period and high carcass weight and quality, important production diseases developed which certainly deserves our attention. Therefore the organizing committee took the initiative to arrange a second session where the problems concerning pale-, soft- and exudative meat and the ability of pigs to move -the latter general indicated as "leg weakness"- could be discussed. In doing so we interested a larger group of investigators and more scientific disciplines who have in common the final goal: the economic production of animal protein of highest quality. On our way to this goal we meet the geneticist, the physiologist, the biochemist, botanist, feed technologist, the nutrionist and many other scientific disciplines. Of course we are aware that we have created these problems ourselves. During the past 30 years extraordinary progress has been made in the development of high-yielding crop plants and animals, with the result that the agricultural production curve has gone steadily upward both in total output and in yield per land unit.
During this period careful attention has been paid to the nutrition of crop plants and domestic animals in order that they might flourish and produce in the service of mankind. As far as the animal production was concerned the troubles started when the supply of nutrients in quantity and/or quality was inadequate for growth, pregnancy or lactation. Nowadays we have to maintain or even increase the production with less labour, more mechanisation, better feed and selected animals.
Speaking of selection of animals we have to find parameters which enable us to exclude already in an early stage of life those animals which likely not will produce profitable under the present environmental conditions. In searching for parameters we need to intensify the study of the homeostatic mechanisms in our farm animals in order to understand why the metabolism fails sometimes. The need for the extension of basic physiological knowledge in solving production disease problems, has encouraged the organizing committee to invite speakers to present the newest data on normal metabolism and on the progress of our knowledge on the nervous and hormonal regulation processus which maintain homeostasis and prevent disorder diseases. The physiologists have received indispensible help from the chemists who developed analytical methods for the isolation and identification of organic compounds in quantities of nanograms and picograms. Among these compounds are hormones, enzymes, releasing and inhibiting factors secreted in the hypothalamus, and factors like somatomedin and somatostatin of which the precise production sites are not completely localized. In tracing these factors and their functions in the metabolism we gradually enhance our insight on basic processus of material production. Sooner or later the new detected factors are synthesized, and some of them or their analogues will contribute to an increased and a more efficient production. Others could be included in the metabolic profile test in order to diagnose in an early stage the incidence of metabolic disorder of subclinical

[1] Parturient hypocalcaemia, J.J.B. Anderson ed., Academic Press, New York, 1970.

[2] Production disease in farm animals, J.M. Payne, K.G. Hibbitt, B.F. Sansom eds., Baillière Tindall, London, 1973.

type. In cattle the most significant gain in the efficiency of output to date has resulted from the intensification of the energy input to dilute the energy cost of maintenance. This has occurred chiefly as the result of increasing the proportion of concentrates to forage fed, of improving the nutritive value of forages and increasing the number of feeding times. Such practices have been dictated by the present economic situation and are expected to continue as long as they are economically feasible. The need for intensification of the energy input has challenged the nutritionist to compose diets with physical and chemical properties which stimulate the organic dry matter intake. Besides, the physiologists were forced to pay attention to animal factors with respect to appetite and feed intake. Many animal scientists have given considerable effort to unravel this regulation system. In studying the literature it is evident that many factors,physical as well as metabolic,are responsible for regulating the intake of food by ruminants and monogastric animals. From the neuro-physiologist we learned the involvement of the hypothalamus in the long- and short term regulation, the localization of receptors in the body and the nature of some signal metabolites. From the experimental parasitologist we learned that the development of a moderate parasitic infection in cows may cause a loss of appetite for a period of several months, even without the occurrence of diarrhoea. The feed intake of mixed concentrates in these cases is unchanged, but the consumption of hay is significantly lower. Obviously an apparant slight damage of the mucosa and submucosa lining the gastrointestinal tract results in a temporaly reduced intake of roughages. In my opinion a thorough study especially of the function of the abomasum and abomasal mucosa might be significant with regard to the short-term regulation of feed intake. For our farm animals, starting with poultry, an environment was created where the highest production was achieved against lowest costs. Close confinement may also pose some social problems for animals. Ulcers, biting, fighting, nervousness and similar problems may be aggravated or even related to the stress of confinement. We must realize we are making animals adapt to the way we want them to live and eat, with less consideration for the fact they are complex living bodies. Behaviour studies will help us to prevent production diseases provoked by the present method of animal husbandry. In the closing session of this conference addressed to all participants we like to consider the stress provoked by the environment which might create social problems for the animals and ethical problems for producer and consumer.

The problems in this field are difficult and challenging but the rewards are great. Among these are the availability of meat, milk, eggs and other animal products of high quality which mankind need for survival. This International Conference has aimed to contribute its share in the development of better production methods in a better world.

Thanks are due to the managing board of the Agricultural University for their generous financial support they gave us in the year we celebrate 100 year teaching Agriculture in the Netherlands.
We gratefully mention the contribution of the Commodities of Feedstuffs and Cattle and Meat as well.

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NEUROENDOCRINOLOGY: COOPERATION OF TWO COORDINATING SYSTEMS

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In a discussion of production diseases it is essential that one establishes the reasons why the normal homeostatic mechanisms have failed. It is, therefore, necessary to understand how the environment impinges on the milieu interieur.

As an endocrinologist I, of course, am convinced that the principal homeostatic mechanisms are endocrine or neuroendocrine. Figure 1, which is modified from a diagram proposed by Follett (1973) and one by Labrie et al. (1975), illustrates what the principal pathways are by which the external stimuli affect the endocrine system. I will use this as a framework for discussion.

There are certain aspects of the improvement in animal production efficiency which I will not discuss because they are outside my area of particular interest and because they would broaden this discussion too much. I will, therefore, assume that nutrition is optimal and I will not discuss the progress in production efficiency as the result of genetic improvement.

Figure 1 shows how various environmental factors via their appropriate receptors are integrated in the brain. In most animals the interaction between the photoperiod and the animal's biological clock is of considerable importance. As a matter of fact, this offers a number of possibilities of improving production efficiency.

In birds there is convincing evidence that the stimulatory effect of long photoperiods on reproduction is mediated via an extra-retinal receptor (Menaker, 1971; Homma and Sakakibara, 1971) and that information via the classical visual pathway, i.e. retina-optic nerve, is ignored (Menaker, 1971). There is no good evidence that such an extra retinal receptor is either present or plays a significant role in mammals (see Menaker, 1971) and the retinal receptor pathway is thus either the only one or the principal one. The extra-retinal receptor according to Oliver and Baylé (1976) is located in the preoptic-suprachiasmatic area of the hypothalamus of Japanese quail (Coturnix c. japonica) and according to Yokoyama et al. (1976) in the infundibular nucleus of white-crowned sparrows (Zonotrichia leucophrys gambelii).

Light can have its effect on physiological functions in two different manners: On the one hand light can synchronize the animal's own endogenous circadian rhythm, or on the other hand light can stimulate certain physiologic functions such as reproductive activity in many species of birds including the domestic duck, the fowl, and the turkey (Van Tienhoven and Planck, 1973). Let us first consider light as a synchronizer.

Halberg (1960) has demonstrated that a great many physiological functions show daily variations. One could make use of such variation to feed animals during the time(s) of the 24-hour period when the response is optimal.

In the case of the malignant hyperthermia syndrome (MHS) in man and pigs (Lister et al., 1975), one could establish the time of day and night. For example, in one strain of mice at 40 weeks of age the percentage of audiogenic convulsions varied between 0 and 15 percent during the day and between 40+ and 60 percent during the night. If it were found that the greatest resistance for MHS occurred at an "impractical time" such as midnight, then one could phase-shift the lights so that the animals consider it midnight, but it is 6 a.m. sun time.

The stimulatory effect on reproduction has been most convincingly demonstrated in a number of bird species in which long photoperiods are stimulatory as they appear to be in the horse (Burkhardt, 1947; Sharp et al., 1975) and the mink (Pearson and Enders, 1944).

Abbreviations - ACTH - adrenocorticotrophic hormone; CRF - corticotrophin releasing factor; DA - dopamine; FSH - follicle-stimulating hormone; GH - growth hormone; GH-RH - growth hormone releasing hormone; GH-RIH - growth hormone-release inhibiting hormone - Somatostatin; GTH - gonadotrophin; GTH-RH - gonadotrophin-releasing hormone; 5-HT - serotonin; LH - luteinizing hormone; NE - norepinephrine; Prol - prolactin; PRH - prolactin releasing hormone; TRH - thyroid stimulating hormone-releasing hormone; TSH - thyroid stimulating hormone.

However, in certain breeds of sheep decreasing day length is necessary to bring them in reproduction (Clegg and Ganong, 1969; Pelletier and Ortavant, 1970). Robinson et al. (1975) produced 3.5 lambs per ewe per year by obtaining an additional lamb crop after manipulation of the photoperiod. The day length was increased abruptly to 18 hours at 60 days of gestation and subsequently reduced 3.5 min per day starting at 90 days of gestation. Progestagens were used to facilitate estrus and ovulation.

Presumably the sensitivity of the hypothalamus-pituitary-gonadal system to the light stimulus shows daily variation. By lighting birds at the time when they are sensitive, one can reduce the total amount of daily light exposure as demonstrated by Follett and Sharp (1969) for male Japanese quail. We have been able to reduce the total of light exposure to four hours per day 2L:12D:2L:8D and 2L:10D:2L:10D without adverse effects on egg production, feed efficiency or shell quality (van Tienhoven and Ostrander, 1976). The savings in energy amount to about 7-10 cents per hen per year, so on a large farm this can be of considerable economic importance. Dufour and Bernard (1968) have shown that pigs raised with one hour of light per day not only showed good gains and feed efficiency, but also showed earlier estrus and no effect on ovulation rate. Clearly the possibilities of reducing energy consumption exist also in this species.

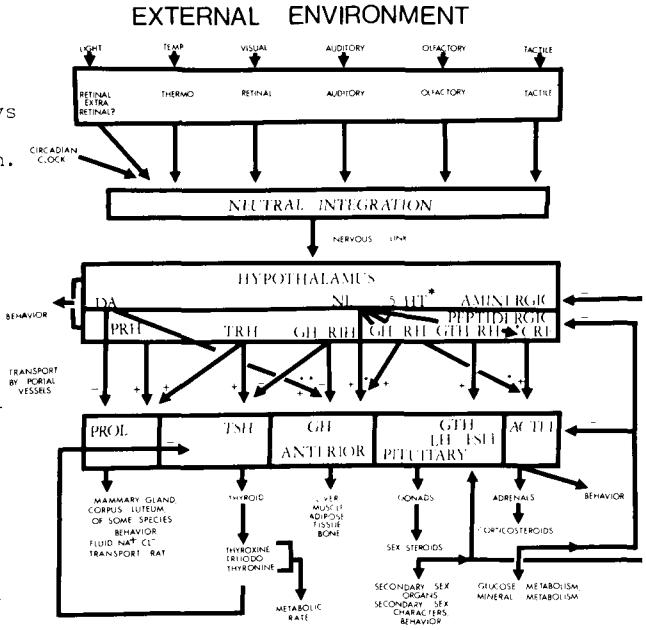
Temperature and humidity can be controled for animals kept indoors and I know of no method by which one can "make use" of temperature fluctuations except for a possible interaction between photoperiod and temperature fluctuations so that the animals' maximal resistance to heat stress occurs when temperatures become high in the pig barns.

The auditory, acoustic, olfactory and tactile stimuli can be manipulated to yield optimal reproductive efficiency as has been demonstrated beautifully by Signoret (1970) and Signoret and Bariteau (1975) for pigs and by Signoret (1975) for sheep. The possibility of a positive feed-back mechanism which operates to a certain degree outside the animal is suggested by the experiments by Macrides et al. (1975) with mice. Exposure to a strange female increased the androgen secretion in the male, making it possible that androgen-dependent pheromones production increases making the male more effective in inducing estrus (Whitten, 1956) and earlier onset of puberty (Bronson, 1975). We can thus, by manipulating the environment, attempt to optimize productive efficiency.

As figure 1 shows the information from the environment is integrated and nervous signals are transmitted to the hypothalamus.

The hypothalamus is, of course, known to play an important role in the regulation of appetite (Balagura, 1970), water intake

Figure 1. Diagram illustrating interaction of environmental factors and neuroendocrine system. Adapted from Follett, 1973, and Labrie et al, 19



*, **, Footnotes under Table 1.

(Fitzsimmons, 1970), body temperature (Richards, 1973), and the cardiovascular system (Zanchetti, 1970).

An interesting development in inducing hyperphagia in geese, and thus obtain the desired pâté de foie gras, has been the intraventricular injection of 6-hydroxydopamine, which destroys the norepinephrine system (Auffray et al., 1973).

The development of the concept of neurosecretory control of pituitary function has been reviewed by Knowles (1974). Neurosecretory neurons share the properties of conventional neurons such as resting potential, action potential but, in addition they secrete neurohormones which are transported via the hypophyseal portal vessels to the pituitary. The neurohormones may be delivered in a number of different ways to the portal bed according to Scott et al. (1974) e.g.: 1) by tubero-infundibular input to the portal bed, 2) entry of the neurohormones via axon-collaterals from hypothalamic or other neurosecretory nuclei into the cerebrospinal fluid (CSF). The tanycytes of the median eminence provide the important link for the transfer from the CSF to the portal system. This system, incidentally, may provide the mechanism for an ultrashort feed-back loop in which neurosecretory hormones which have entered the CSF may act back on the neurosecretory neurons.

The neuroendocrine hormones can thus act as translators, between the nervous and the endocrine system as E. Scharrer has pointed out. One can classify these neurohormones into two

major groups, i.e. the aminergic and the peptidergic neurohormones.

Among the aminergic hormones we find norepinephrine, epinephrine, dopamine and serotonin. These amines are chemically identical with the neurotransmitters of the same name but they differ functionally from them in important aspects (Scharrer, 1969). As neurohormones they are transported by the blood instead of being taken up again as e.g. norepinephrine is as a neurotransmitter. Morphologically the neuroendocrine cells seem to have a higher content of biogenic amines and the vesicles, indicating the presence of these amines, are present in all parts of the neuroendocrine neuron (Scharrer, 1969), but they are found in the presynaptic part in neurotransmitter neurons.

A number of the peptidergic neurohormones have been isolated, their structure determined and their laboratory synthesis worked out. Table 1 illustrates the structures of some of these hormones.

This knowledge has made it possible to obtain a better understanding of the mechanism of action (Labrie et al, 1975) and to synthesize releasing factors e.g. LH-RH 20 times more potent than the natural hormone (Coy et al., 1975), and inhibitors of these hormones e.g. LH-RH (Coy et al., 1975).

These synthetic releasing hormones and their analogs can be used in a number of different ways to regulate ovulation. One might expect that these neurohormones would have fewer side effects than for instance steroid hormones because presumably the pituitary would be the specific target organ and one might expect a great dilution when the neurohormones reach the general circulation and they might possibly be metabolized rapidly. However, as has proven to be the case quite often, there were some surprises in store.

It has been found that somatostatin inhibits not only the release of growth hormone, but also inhibits the secretion of insulin and glucagon in vivo (Labrie et al., 1975). The inhibition of glucagon, which is produced in the pancreas and in the gut, has made it possible to prevent insulin-deficiency hyperglycemia by somatostatin treatment (Gerich et al., 1974). It now appears that somatostatin may not be produced by the brain only, but also by the pancreas and the stomach (Arimura et al., 1975).

In a recent experiment Kerdelhué et al. (1976) found that a single i.p. injection of LH-RH antiserum to female rats at noon of proestrus resulted not only in the expected block of LH and FSH release, but also in a long lasting hyperprolactinemia, and persistent estrus with large cystic ovarian follicles and high plasma estradiol concentrations. The hyperprolactinemia is probably the result of abnormal ovarian function. Similarly, Arimura and Schally (1976) have reported that passive immunization of rats with an antiserum to somatostatin resulted in a 250% elevation in basal serum TSH and a doubling of the response to TRH. Such experiments should act as warning signs with respect to the application of antisera to hormones in animal management and human medicine.

Neurohormones also affect behavior. Systematic LH-RH injection, for instance, facilitates the effect of estrone in inducing lordosis in ovariectomized and ovariectomezed-adrenalectomized rats, but LH, FSH and TRH do not have this effect (Moss et al., 1975), but LH-RH does not advance the onset of sexual receptivity in intact rats. Infusion of LH-RH into the medial preoptic area of estrone-primed, ovariectomized rats had a slight facilatory effect on lordosis behavior, whereas infusion into the ventromedial-arcuate complex did not.

In male rats LH-RH reduced the time to first intromission and ejaculation, but had no effect on the number of mounts, intromissions or lordosis/mount ratios. In castrated-testosterone-primed rats LH-RH reduced the time to achieve ejaculation (Moss et al., 1975).

TRH has been reported to have an antidepressant effect in some humans (Prange et al., 1975), to have beneficial effects in some forms of schizophrenia (Prange et al., 1975), but to induce shaking behavior in rats when injected into the hypothalamus or the thalamus (Wei et al., 1975). This shaking behavior resembles that observed after morphine withdrawal. TRH antagonizes the sedative effects of barbiturates, chloralhydrate and reserpine. These effects are observed in intact and/or hypophysectomized mice, apparently by an effect other than promotion of norepinephrine activity (Prange et al., 1975).

Somatostatin causes a reduction of spontaneous activity; it prolongs phenobarbital sedation and exaggerates hyperthermia (Prange et al., 1975).

M.I.F. may have an anti-Parkisonian activity (Kastin et al., 1975) in humans, and it induces stereotyped compulsive behavior in unrestrained cats.

These effects of neurohormones on behavior are not surprising in view of the findings that these hormones affect the activity of individual neurons. LH-RH, for instance, increases the activity of units in the medial basal hypothalamus (Kawakami and Sakuma, 1974). On the other hand, TRH, LH-RH and somatostatin decreased the discharge frequency of neurons in the ventromedial hypothalamus, the cuneate nucleus and the cerebellar cortex (Renaud et al., 1975). LH-RH and TRH have also been reported to decrease the firing rate of the neurons in the preoptic area - anterior hypothalamus (Dyer and Dyball, 1974).

The pituitary hormones LH and FSH also can change the activity of neurons in the medial basal hypothalamus and medial preoptic area (Kawakami and Sakuma, 1974). The effects of neurohormones, pituitary hormones and steroids on activity of the CNS thus suggest the presence of ultrashort, short and long feedback mechanisms in the CNS.

A discussion of the specific effects of pituitary and of steroid hormones on behavior would require much time and space and you are familiar with the principal effects.

It needs to be pointed out that the gonadal steroid hormones can affect the response of the pituitary to administered releasing hormones (Cooper and McCann, 1975; Fink et al., 1975) while GTH-RH also has a priming effect on the pituitary for gonadotrophin release (Fink et al., 1975).

An important link between estrogens on the one hand and the catecholamines on the other hand has been suggested by the work of Breuer and Köster (1975). Hydroxylation at the C_2 position is an important step in the metabolism of estrogen. These 2-hydroxy-estrogens have a catechol structure and inhibit the methylation of catechol amines by the enzyme catechol - O methyltransferase (COMT). This interaction between the metabolic pathways of the two coordinating systems suggests how estrogens may have their effect on the CNS. Recently Parvizi and Ellendorff (1975) have shown that injection of 2-hydroxyestradiol into the amygdala of miniature barrows decreased the plasma LH concentration.

The correlation between norepinephrine content of the hypothalamus of rats (Sandler, 1968; Stefano and Denoso, 1967; Lichtensteiger, 1970) and the stages of the estrous cycle suggest that estrogen may cause an increase in norepinephrine concentration and thus may increase LH-RH secretion. It is, of course, also possible that the correlation is not a causal one. The observation that the nuclei of certain hypothalamic cells show the presence of radioactivity after the injection of ^3H - estradiol and that these same cells show the presence of catecholamine fluorescence (Grant and Stumpf, 1974) lends support to the speculation of an intimate relationship between steroid hormones and the aminergic neuroendocrine system.

The scheme outlined in Figure 1 is an oversimplification, but it provides a framework within which one can formulate experiments that need to be carried out to improve productivity, improve reproduction and reduce production diseases and at the same time make animal husbandry more economical.

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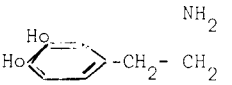
Summary of the discussion

Queries were made as to details of the given schemes for transport of impulses along the hypothalamus-pituitary gland, the further endocrine system and the gut on the other hand. The effect of the length of dark and light periods was discussed in some detail. It was suggested from the auditorium that more information could be gathered by studying the length of the gestation period in wild animals subjected to large extremes in the amount of daylight, for instance free flying fowl or reindeer.

Table 1. Structure of Some Hypothalamic Releasing and Inhibiting Hormones.

TRH-TSH-RH	= Pyro-Glu-His-Pro-NH_2
LH-RH	= Pyroglu-His-Trp-Ser-Tyr-Gly-Len-Arg-Pro-Gly-NH_2
GH-RH	= Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala
GH-RIH (somatostatin)	= Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH
MIF[1]	= Pro-Leu-Gly-NH_2
	Cys-Tyr-Ile-Asn-Cys-OH
MRF[2]	= Cys-Tyr-Ile-Gln-Asn-Cys-OH
PIF	= 
Dopamine	

[1]MIF - Melanophore Stimulating Hormone Inhibiting Factor

[2]MRF - Melanophore Stimulating Hormone Releasing Factor

Reference: L. Martini, 1974.

*It is not firmly established whether these biogenic amines act directly on the anterior pituitary or via the peptidergic neurohormones. For the effect on ACTH release see Ganong (1975), for GH release see Müller and Cocchi (1974).

**Effect may be species dependent (Müller and Cocchi, 1974).

ABNORMAL LIPID METABOLISM AND PRODUCTION DISEASES

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Introduction

When a biochemist gets mixed up with people involved in animal production, animal nutrition and similar applied disciplines, he is always left with the feeling that the biochemical theory is lagging far behind the empirical progress made in these more practical fields of research. This is not a new observation:

- Vitamins were discovered and vitamin deficiencies were described and cured long before anything was known about vitamin action at the molecular level in the living organism.
- The clinical disorder of cattle, known as grass tetany, was linked with hypomagnesaemia around 1930 and has since been cured by provision of extra dietary magnesium, but still it is completely unknown which biochemical systems are upset, which enzymes suffer from the magnesium deficiency and what metabolic disturbance gives rise to the well-known clinical symptoms.

Again, going over the abstracts of the papers to be presented at this meeting, I experienced the same feeling. Basic science in general, and fundamental biochemistry in particular, fail to supply an adequate theoretical framework in support of your experimental work. The reverse statement is equally true, but it sounds less agreeable: For the greater part, your work is practical to such an extent that you do not even seem to bother about the lack of a scientific foundation. Only in exceptional cases a molecular mechanism can be brought forward to link the observed symptoms of a production disease to known primary lesions, conditions or deficiencies. Only in exceptional cases a biochemist is rejoiced at recognizing a known pattern of metabolic disturbances. It is about some of this rejoicing that I will tell you today.

Acetonaemia

Ruminant ketosis is the production disease which can be most completely described in terms of the underlying biochemical processes and control mechanisms. We can produce a fairly comprehensive picture of the chain of events leading from the primary cause of the disease to the observed biochemical symptoms.

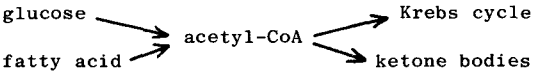
The primary cause of acetonaemia is the increased demand for glucose. In dairy cattle, in which the disease occurs at the peak of lactation, the mammary gland may take up so much glucose that a hypoglycaemia develops. In sheep, it is the fetus, or more often the fetuses, which drain away glucose from the maternal circulation and thereby precipitate the disease which in sheep usually occurs prior to parturition.

Ruminants have an extra difficulty in this respect since virtually no glucose is absorbed from the gut, making the animal completely dependent on glucose synthesis, so-called gluconeogenesis, in liver and kidneys.

It is often stated that the direct cause of the disease is a lack of energy. This is an old misunderstanding which is difficult to wipe out. It is now perfectly clear that the primary defect is the drain of glucose and the inability of the organism to meet the demands. A number of conditions is known in a large variety of animals, including man, in which the availability of glucose is decreased. These conditions range from diabetes mellitus via fasting, hungering and living on a high-fat, low-carbohydrate diet, all the way to "spontaneous" acetonaemia in ruminants. In all these conditions, independent of energy considerations, the decreased availability of glucose by itself leads to the development of ketosis or, in biochemical terms, to an hepatic overproduction of ketone bodies.

Regulation of ketogenesis

Why does a decreased availability of glucose bring about an increased rate of ketogenesis? This question is often answered by saying that the shortage of glucose makes the liver switch over from glucose breakdown to the oxidation of fat and that the latter process necessarily brings about the increased production of ketone bodies. However, this explanation is too simple: Ketone bodies are produced from acetyl-CoA which is not only formed during the oxidation of fatty acids but is also an intermediate in the breakdown of glucose.



It is clear then that the key to the control of ketogenesis is in the regulation of the metabolism of acetyl-CoA. The question, therefore, arises: Why is acetyl-CoA during fatty acid breakdown mainly converted into ketone bodies, whereas it is mainly oxidized in the Krebs cycle during glucose breakdown? There are at least three answers to this

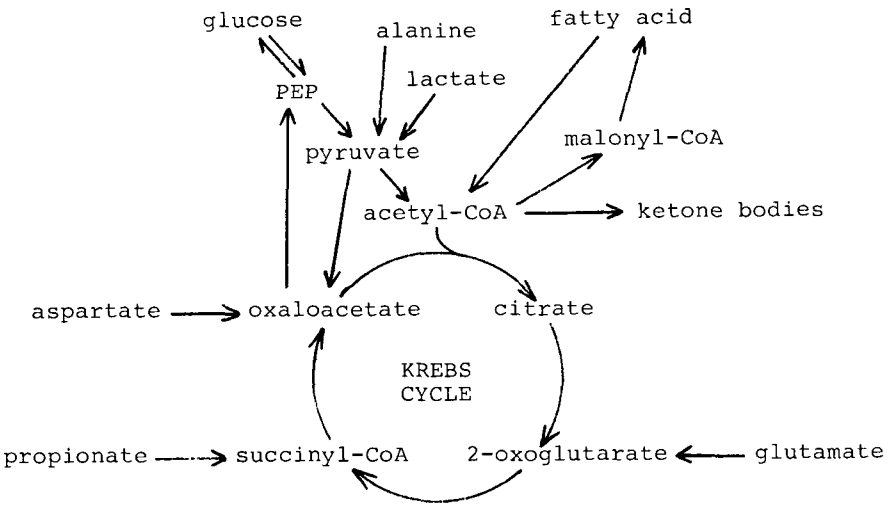


Fig. 1. Principal pathways of metabolism of glucose and fatty acids in the liver.

question:

1. The capacity of the Krebs cycle to oxidize acetyl-CoA is to a large extent determined by the amount of oxaloacetate which is available to condense with acetyl-CoA to form citrate (Fig. 1). During glucose breakdown oxaloacetate may be formed from pyruvate by the action of pyruvate carboxylase and thereby, the activity of the Krebs cycle may be increased. On the other hand, fatty acid oxidation cannot increase the amount of Krebs-cycle intermediates.
2. During glucose breakdown part of the acetyl-CoA is used for fatty acid synthesis. The acetyl-CoA is first converted into malonyl-CoA by the enzyme acetyl-CoA carboxylase. Malonyl-CoA then condenses with an acyl-CoA ester to lengthen the hydrocarbon chain of the latter by two carbon atoms (see Fig. 5). Synthesis of fatty acids does not occur during fatty acid oxidation. In general, a cell always resists to the occurence of such "futile cycles". In this particular case, the key enzyme of fatty acid synthesis, acetyl-CoA carboxylase, is inhibited by the increased level of long-chain acyl-CoA.
3. During shortage of glucose, the liver will try to maintain glucostasis by increasing the rate of glucose synthesis from non-carbohydrate precursors. One of these precursors is oxaloacetate which can be converted into phospho-enolpyruvate (PEP) by the action of PEP carboxykinase (Fig. 1). In conditions of active gluconeogenesis, the capacity of the Krebs cycle is, therefore, further decreased.

Gluconeogenesis

Oxaloacetate is a key intermediate in gluconeogenesis. Since the glycolytic conversion of PEP into pyruvate is irreversible, gluconeogenesis from pyruvate occurs via oxaloacetate (Fig.1). The same is true for gluconeogenesis grom alanine and lactate. In fact, all compounds which can be converted into oxaloacetate can be used as gluconeogenic precursors. This is shown in Fig. 1 for the amino acids glutamate and aspartate and for propionate.

In ruminants, propionate is a very important substrate for gluconeogenesis. By the reaction sequence shown in Fig. 2 it is converted into succinyl-CoA which is an intermediate of the Krebs cycle.

Fat is the main energy store of the mammalian organism and would, therefore, be the most obvious source of substrate for gluconeogenesis. However, although animals can convert carbohydrate into fat, the reverse process cannot occur. This is caused by the irreversibility of one single reaction step: the conversion of pyruvate into acetyl-CoA. The low CO_2 pressure in the cell prevents the reversal of this reaction catalysed by the pyruvate dehydrogenase complex. The observation that some label may eventually appear in glucose if 14-C-labelled fatty acids are administered to liver preparations does not alter the conclusion that no net conversion of fatty acids into glucose can occur. This observation is explained by the fact that some oxaloacetate may escape from a labelled pool of Krebs-cycle intermediates.

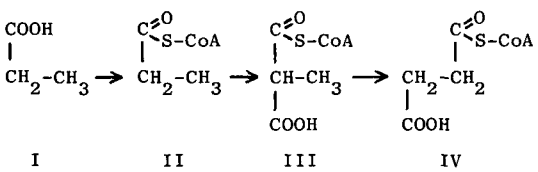


Fig. 2. Conversion of propionate (I) into succinyl-CoA (IV) via propionyl-CoA (II) and methylmalonyl-CoA (III).

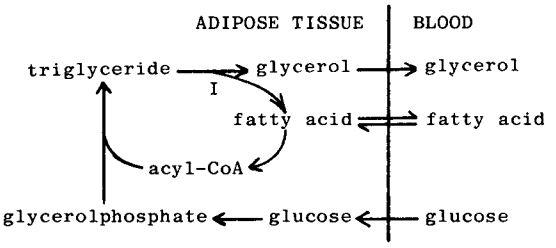


Fig. 3. Turnover of triglycerides in the adipose tissue. I: the hormone-sensitive triglyceride lipase.

Adipose tissue

A very simple metabolic control mechanism is responsible for the increased level of plasma free fatty acids, observed in all conditions of a lowered glucose availability. In the adipose tissue a constant turnover of the triglycerides takes place (Fig. 3). Triglycerides are broken down to glycerol and fatty acids; they are resynthesized from glycerolphosphate and the coenzyme A esters of the fatty acids. The enzymes which will convert the fatty acids into their coenzyme A esters are present in the adipocyte, but the enzyme glycerokinase, which should phosphorylate glycerol, is absent. The glycerolphosphate which is necessary for triglyceride synthesis in the adipose tissue is derived from glucose. A lowered availability of glucose will thus lead to a decreased triglyceride synthesis. At a constant rate of triglyceride hydrolysis this will bring about an increased release of fatty acids from the adipose tissue and an increased plasma level of albumin-bound non-esterified fatty acids.

Undoubtedly, other factors are also operative in the regulation of adipose tissue lipolysis. The activity of the enzyme triglyceride lipase in the adipose tissue is controlled by several hormones and, moreover, the rate of fatty acid release is dependent on the degree of saturation of plasma albumin with fatty acids (Metz et al., 1973). But the simple mechanism outlined above is sufficient to explain the effect under discussion.

Ketogenesis: an overflow process

It has been demonstrated conclusively that the uptake of glucose and fatty acids by the liver is directly proportional to their concentration in the plasma. A decreased availability of glucose and the resulting higher plasma fatty acid level will together bring about a switch-over of the liver from glucose to fatty acid utilization. As described above, this switch-over is accompanied by an increase in ketogenesis.

It is clear that all nutritional and hormonal factors affecting fatty acid mobilization from the adipose tissue, will influence ketogenesis accordingly. In this connection, it is interesting that 3-hydroxybutyrate was found to inhibit lipolysis in adipose tissue (Metz et al., 1974); this ketone body thereby establishes a feed-back loop over the whole range of events leading to ketosis.

Lopes-Cardozo and Van den Bergh (1974) have demonstrated that ketogenesis may be described as an overflow of acetyl-CoA from the Krebs cycle. Acetyl-CoA is preferentially taken up in the Krebs cycle. Only when the rate of acetyl-CoA production increases beyond the capacity of the Krebs cycle, acetyl-CoA is converted into ketone bodies (Fig. 4).

Physiological function of ketogenesis

Ketogenesis is often regarded as a kind of hepatic dysfunction, as a pathological process per se. Yet, it is clear that such a quantitatively important process must have a physiological function.

For the liver, the ketone bodies are end products; they are circulated to the extrahepatic tissues, most of which have a large capacity for their oxidation. Under physiological conditions the production of ketone bodies in the liver is balanced by their utilization in the extrahepatic tissues. Whenever the availability of glucose for the organism is depressed, the liver will increase its rate of ketogenesis and many tissues will respond and switch over to use ketone bodies as a quantitatively important source of energy. In such conditions, increased plasma levels of ketone bodies are observed: a physiological ketosis develops.

A pathological ketosis may develop when the production of ketone bodies exceeds their utilization. The level of ketone bodies in the blood will increase until a balance with renal excretion is reached.

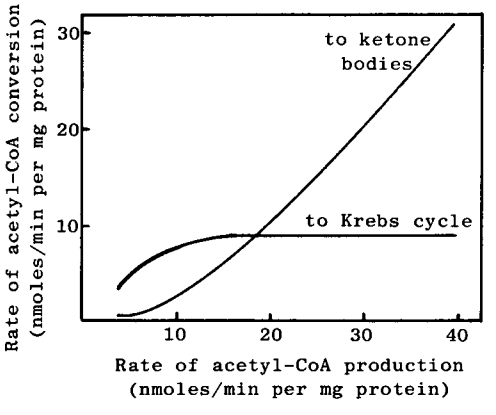


Fig. 4. Relationship between the rate of acetyl-CoA production in β-oxidation and the rates of entrance of acetyl-CoA into its main metabolic pathways. The rate of palmitate oxidation by rat-liver mitochondria was varied as described by Lopes-Cardozo and Van den Bergh (1974).

Ketone bodies play an important role in "caloric homeostasis" (Krebs et al., 1971). The increased ketogenesis in the liver serves the same purpose as the increased mobilization of fatty acids from adipose stores: to provide the tissues with an energy source to replace glucose. At first sight, the presence of two alternative fuels for the replacement of glucose seems redundant. In recent years it has become clear, however, that the primary function of ketone bodies, which cannot be fulfilled by fatty acids, is to provide the brain with a readily oxidizable substrate when the availability of glucose is depressed. The brain has an extremely low capacity for the oxidation of fatty acids. The enzymes involved in ketone-body utilization are present in the brain of a large variety of animals, both vertebrates and invertebrates (Williamson et al., 1971; Sugden & Newsholme, 1973). The plasma level of the ketone bodies is the only factor controlling their rate of utilization in the adult brain (Williamson et al., 1971). It has been calculated that up to 50% of the energy demand of the brain can be covered by oxidation of ketone bodies (Hawkins et al., 1971).

Therapy of ruminant ketosis

Since so much is known about the metabolic disturbances underlying acetonaemia, it is not difficult to understand most of the vast number of antiketogenic treatments. These must all be directed towards an increased availability of glucose to the organism. This can be brought about by increasing:

a) the absorbtion of glucose from the gut;
b) the supply of gluconeogenic precursors;
c) the activity of the gluconeogenic enzyme system.

a) Oral administration of glucose is obviously useless since rumen fermentation will break down the glucose before it can be absorbed. It has been proposed that glucose might be supplied intravenously, subcutaneously or even rectally, but the required quantities (up to 2 kg/day) are so huge that this treatment is unpracticable. Moreover, as a therapy it would have the disadvantageous side-effect that gluconeogenesis would be depressed as a result of the artificially increased plasma glucose level. In this way the animal would remain dependent on the treatment.

In cattle and sheep on high-concentrate diets, some carbohydrate may excape fermentation in the rumen and some glucose may be absorbed from the intestines. To my mind, more research should be directed towards the production of "protected" carbohydrates, covered by an artificial layer which will prevent fermentation in the rumen, but which may be digested later in the gastro-intestinal tract.

b) In order to increase the supply of substrates for gluconeogenesis, propionate has been added to the diet of cattle and sheep. The treatment indeed has some beneficial antiketogenic effect. However, one should always be aware that the therapy of one disease may immediately induce another production disease.

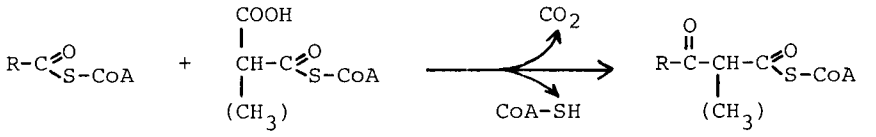
A remarkable effect of the addition of propionate to the diet of lambs was observed by Garton et al. (1972). These authors noticed that the subcutaneous adipose tissue of lambs given a propionate-containing diet over a 20-week period was unusually soft. The triglycerides of this soft adipose tissue were characterized by the presence of large proportions of a wide variety of odd-numbered as well as monomethyl branched-chain fatty acids. It was concluded that the softness was associated with the lower melting point of branched-chain acids as compared with their straight-chain analogues.

From a biochemical point of view, these observations are easily explained. The pathway of propionate metabolism is depicted in Fig. 2. With an increased supply of propionate, it may be expected that the concentrations of all the intermediates of this pathway are elevated. Since propionyl-CoA is the key substrate for the synthesis of odd-numbered fatty acids, an enhanced availability of this compound may explain the increased amounts of odd-numbered acids.

The effect of an increased level of methylmalonyl-CoA is more unexpected. From the presence of the monomethyl branched-chain fatty acids in the triglycerides it must be concluded that methylmalonyl-CoA accumulated to such an extent that it could take the place of malonyl-CoA in fatty acid synthesis and thereby give rise to the branched-chain fatty acids (Fig. 5).

In order to increase the supply of propionate, it is not necessary to add it to the diet. Rumen fermentation may be manipulated in such a way that larger proportions of propionate are produced. A number of therapeutics commonly used against ketosis in dairy cattle stimulate propionate production in the rumen. The best-known example is the antiketosis drug chloralhydrate. Its mechanism of action was elucidated independently in Gent (Van Nevel et al., 1969) and in our laboratory in Utrecht (Prins, 1970). The primary effect of chloralhydrate is the inhibition of the production of methane leading to an accumulation of hydrogen in the rumen. Part of this hydrogen is used for an increased production of propionate from pyruvate.

c) The antiketogenic action of glucocorticosteroid administration is largely explained by the fact that these hormones induce the synthesis of some key enzymes active in gluconeogenesis from amino acids, e.g. various transaminases, pyruvate carboxylase and fruc-



acyl-CoA (methyl)malonyl-CoA (branched-chain)3-oxoacyl-CoA

Fig. 5. If methylmalonyl-CoA accumulates as a result of an increased metabolism of propionate, it may take the place of malonyl-CoA in fatty acid synthesis and thereby give rise to branched-chain fatty acids.

tose-1,6-diphosphatase (an enzyme involved in the conversion of PEP into glucose).

Ketosis in pigs

To what extent do the processes which we have discussed so far apply to porcine metabolism? Searching the literature for disturbances of lipid metabolism in pigs, one may easily get the impression that pigs have no liver. Abnormal lipid metabolism in pigs seems to be confined to the adipose tissue. The literature on lipid metabolism and its abnormalities in pig adipose tissue is indeed very extended.

Only after a careful search of the literature, reports were found on the occurrence of ketosis in sows at the peak of lactation (for a review, see Sampson, 1964). Although very few cases are reported, the data produced sound very familiar. Therefore, the story which I have told so far is obviously to a large extent also applicable to pigs.

That leaves us with the question why ketosis in pigs never was as important a problem as it is in cattle and sheep. At least three reasons can be brought forward:

- In contrast to ruminants, pigs can absorb large quantities of glucose from the gut.
- Pigs have a highly active gluconeogenic system. It is almost impossible to make a pig hypoglycaemic by fasting. It is well-known that newborn pigs may develop a fatal hypoglycaemia if the sow does not produce sufficient milk for its litter. In contrast, pigs older than four days can be starved for days without developing hypoglycaemia (Swiatek et al., 1968).
- Pigs have never been selected for high milk production. The disease does not occur because you have not produced it.

And that seems a beautifully appropriate statement to leave the further discussion to you.

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Summary of the discussion

Some consequences of these schemes, particularly regarding the conversion of oxaloacetate and the coupling to the citric acid cycle, were further discussed. Glucocorticosteroids will remove the main cause of acetonemia by depressing milk production. Increase of the citrate synthetase activity would not have the expected beneficial effect, since in the citrate synthesis not the enzyme but oxaloacetate is the rate limiting factor. Glucocorticosteroids increase the total glucose production in sheep. In studies with ^{14}C-labelled amino acids and propionate, the latter did not seem to be a precursor of glucose, possibly so because of insufficient supply of propionate or non-induced enzymes for the conversion after administration of corticosteroids. Ketone bodies are produced in the rumen epithelium in rather constant amounts, hence their inhibition of lipolysis in the adipose tissues will be limited. Treatment with chloral hydrate is not unlawful in the Netherlands.

POSSIBLE WAYS OF FIGHTING ENVIRONMENTALLY EVOKED PRODUCTION DISEASES

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Summary

The disease panorama in cattle, pigs and poultry during the last decades shows considerable changes. The frequency of certain diseases has increased while others have decreased. The increase is most marked in environmental production diseases e.g. mastitis in cows, pneumonia in calves, agalactia and to a certain extent enteritis in pigs as well as traumatic injuries in poultry. The increase in environmentally evoked diseases is mainly due to the changed relationships between the animals and their environment.

The changed animal-environment relationship is due to alterations in phenotype as well as to changed environment. The changes of phenotype are mainly caused by a breeding and feeding directed to increased production. The change in environment is mainly due to specialization in only one type of animal or in only one type of production, larger herds and new techniques in the animal environment.

An effective way of combating environmentally evoked production diseases is necessary for ethical as well as economic reasons. It is not ethically justifiable to buy increased productivity at the price of increased morbidity or increased suffering on the part of the animals. It is also not economically justifiable that the profits - by increased productivity and new techniques - should be counterbalanced by losses through animal diseases.

An effective combating of environmentally evoked production diseases requires some new methods to be used in veterinary medicine which in turn require new knowledge. The increased possibilities for successful therapy of the already sick animal or group of animals should, of course, be utilized and developed, but in order to effectively combat disease, the veterinarians must be able to utilize preventive methods. This requires particular efforts in the individual herds. In many cases it is, however, already too late at the herd level. Efforts must be made at regional or national level in order to combat disease effectively.

In Sweden the combating of environmentally evoked diseases has been rendered more effective by the fact that all plans and drawings for new constructions or alterations of animal houses, must be presented to and approved by veterinarians specializing in animal hygiene. The assessments made so far indicate that this way of fighting disease has been successful at both regional and national levels.

Increased research will be necessary in the future in order to render the combat of environmentally evoked production diseases more effective through preventive methods not only in the single herd but also regionally and nationally.

Introduction

The total disease pattern in cattle, pigs and poultry has changed considerably during the last decades. The frequency of certain diseases has increased while other diseases have decreased. The increase is most marked with regard to environmentally evoked production diseases. This change in the pattern and the frequency of diseases is caused by changed relationships between the animals and their environment. The reasons are changes in animal material as well as in animal environment (Ekesbo, 1973).

Changes in phenotype

The examples of changes in the Swedish animal material accounted for here (Ekesbo, 1975; Redogörelse Svinstamkontrollen, 1974; Svensk Husdjursskötsel, 1970, 1975) should be quite representative of the conditions in most industrialized countries.

While the annual average increase in output in kg milk per cow from the eighteen forties to the end of the nineteen fifties during different periods differed between 6 and 23 kg the annual average increased to around 97 kg between 1958 and 1974. The milk yields for milk-recorded SRB-cows rose during this period by 40 % to 5,430 kg and for SLB-cows by 30 % to 5,760 kg. The body length of fatteners of the Yorkshire breed increased by 4 % from 1955 to 1972, the growth rate from 699 g/day to 750 g/day between 20 and 90 kg while the backfat decreased by 29 % at the same time. The mean egg production per hen increased by 72 % to 5.3 kg from 1955 to 1974. The rearing time for broilers up to 1.5 kg weight was 12 weeks in 1955 and 6.5 weeks in 1974.

Changes in livestock environment

The number of dairy herds in Sweden was 240,000 in 1955 and 68,000 in 1974. The mean number of cows per herd increased from 6.0 to 10.1. The total number of cows decreased from 1,465,000 to 690,000. The number of cattle

herds specializing in meat production increased from a few in 1955 to around 22,000 in 1974. The number of sow herds with more than 20 sows has also increased very considerably. The mean number of sows per herd in the whole country was 3.0 in 1955 and 8.7 in 1974. The number of fattener herds with more than 500 pigs increased considerably between 1955 and 1974 and in 1974 only 4 % of the producers delivered more than 63 % of all pigs for slaughter. The number of poultry herds decreased from 300,000 in 1955 to 40,000 in 1974. 2 % of these accounted for 70 % of the total production.

The structural changes influence the herd size but also lead to specialization in one type of animal or in one type of production. At the same time other environmental changes have taken place e.g. an increase in the noise level due to added demands for ventilation installations or increased dust content in the air due to a combination of heat supply, ventilation and automatic feeding arrangements (Ekesbo, 1973). New techniques with regard to manure handling have influenced the conditions in the animal houses as well as the risks of contaminating the pasture (Jack & Hepper, 1969; Thunegard, 1975).

Animal disease patterns and animal behaviour versus production intensity and changes in the animal environment

While cystic ovaries and paresis puerperalis in cattle seem to be correlated with a production increase, agalactia toxaemica in pigs appears to be related both to production increase and environmental changes (Bäckström, 1973; Henricson, 1957; Jönsson, 1960; Ringarp, 1960).

There are statements to the effect that both the mortality in calves and piglets and the frequency of respiratory diseases in pigs are higher with an increased herd size. Many researchers have shown the advantages of breeding in batches rather than non-stop rearing (Bäckström, 1973; Lindqvist, 1974).

Larger herds, reduction of the freedom of movement of the animals and other environmental factors have been shown to influence the incidence of traumatic injuries (Bäckström, 1973; Ekesbo, 1966; Lindqvist, 1974; Svedberg, 1976; Vilson, 1976).

The introduction of liquid manure handling brought about a change in the panorama of disease in many herds due to the effects of manure gases on the animal organism (Högsved, 1968).

The negative effects of the environment in connection with new methods, e.g. liquid manure handling or large herds, have enforced increased demands for mechanical ventilation. This has caused a higher noise level in many animal houses. It is known that noise can cause different pathological changes in the animal organism (Algers et al.)

The decreased use of bedding that has occurred and still exists in some instances has influenced the panorama of disease in different ways such as increased incidence of traumatic udder injuries and tail biting in pigs etc. (Ekesbo, 1966; Högsved, 1969). Keeping the animals indoors throughout the year, which has become the rule in many types of animal husbandry has resulted in an increased incidence of disease (Ekesbo, 1966).

Swedish investigations indicate that some diseases, mainly those where the symptoms are changed animal behaviour, seem to be more frequent in environments which give the animals a combination of under- and over-stimulation. Examples are abnormal licking in cows, abnormal biting in sows or cannibalism in pigs or poultry in barren environments where the noise levels are high (Ekesbo, 1975). A further example of abnormal behaviour in unsatisfactory environments is a general uneasiness in the animals, revealed e.g. by playing with the water bowls, common in both cattle and pig herds (Ekesbo, 1976). In some countries efforts have been made to control some forms of morbidity which mostly appear in form of changed behaviour by keeping the animals in the dark during most of the 24 hours.

Healthy animals and modern technology are not imcompatible

Modern animal production, where the animals' environmental needs are taken into consideration, does not necessarily mean any change in the pattern and incidence of disease other than that caused by an increased production and changed genotype. A suitably planned loose housing environment for dairy cows is an example thereof (Ekesbo, 1971). In such cases, however, an adaptation of the technical environment to the demands of animal health including behaviour is necessary (Ekesbo, 1973). This aim can be achieved by intensified preventive veterinary medicine measures based on increased research concerning the impact of the environment on the animal health.

Two different types of disease-evoking environmental factors

A systematic study of different factors in animal environment shows them to belong to two main groups, invariable and variable factors. Invariable factors are e.g. the design of the animal building, the stall-length and stall-breadth, size and form of calf and swine pens, ceiling-height, type of ventilation and very often recruiting system. Variable factors are e.g. management, quantity and quality of feeding, temperature in the house, amount of straw, breed of

animals. Invariable factors are very difficult, in practice often impossible, to change in the single herd, variable factors are more or less changeable in the single herd.

Naturally there is no distinct general limit between these two groups. That which in one herd is a variable factor may in another be a definitely invariable one. One example is the amount of straw used as bedding which in most herds is a variable factor. In herds with liquid manure handling straw bedding can definitely not be used and thus becomes an invariable factor.

Environment - animal health - public health

Several environmentally evoked diseases which have increased because of changes in the animal environment will have an increased economic importance if consideration is given to the food hygiene consequences they bring about. Some examples are mastitis in cattle and tail biting and pleurisy in pigs. The combating of these and similar diseases is therefore of importance as far as food hygiene is concerned whether it is done directly by the owner or legislatively at a regional or national level.

An increased incidence of environmentally evoked diseases caused by changed phenotype or changed environment is thus unacceptable, both from the point of view of economy for the owner or with regard to food hygiene for society as a whole or for ethical considerations. The intended gains through genetic or technical advances will then be decreased by losses through animal diseases.

Combating environmentally evoked diseases

It is very easy to say that environmentally evoked diseases must be fought by eliminating the main reasons for the diseases. In practice this is certainly possible when the disease is caused by some variable factors. The veterinarian can for example combat traumatically evoked mastitis in one herd by having the farmer increase the amount of straw for bedding and thus decrease the amount of traumatic udder injuries. However, if the traumatic udder injuries are caused by too short stalls it would not always be possible for the farmer to follow the advice to increase the stall length and thus diminish the incidence of mastitis. Other examples are a high pleurisy incidence in a fattening pig herd caused by too great a number of pigs in the same animal house which is more difficult to change than to change from continuous rearing to rearing in batches in moderate house sizes.

From the point of view of food hygiene, effective disease combating or ethical considerations it is not acceptable to try to compensate a poor environment by medication. One example of this is treatment of a high incidence of pneumonia in calves or pigs with antibiotics when rearing in batches should have been chosen in order to prevent a high incidence of pneumonia. No less unacceptable is combating abnormal behaviour in the form of playing with the water bowls by taking them away and introducing restricted water rationing or trying to fight cannibalism by keeping the animals in darkness. This is no solution of the basic problem and is of the same order as cutting off the pigs' tails to prevent tail biting. In such cases it is of course necessary to attack the primary cause which is neither the water bowls nor the tails but an environment which does not satisfy the most elementary needs of the animals.

The change from traditional animal husbandry to in may aspects new techniques and methods very often present the practising veterinarians with problems. Effective disease combating only in the single herds is often not practicable or at least very difficult to carry through. Experience in Sweden shows that during the sixties high disease incidence very often occurred in herds where new techniques or new methods were used. Investigations have revealed that the diseases in such cases have as a rule been environmentally evoked. As in many cases the disease-evoking factors are invariable it is difficult for the practising veterinarian to carry out effective disease-combating in the single herd. In order to solve its primary task, to combat disease, veterinary medicine therefore must enter upon a new course of thinking as far as the environmentally evoked diseases areconcerned. This means an increased concentration on research within the field animal environment - animal health followed by new methods for disease-combating.

The new methods for disease-combating must imply that disease-preventive measures are put into practice at the same time as new animal projects are planned.

In Sweden this new frontline in disease-combating is opened through local, regional and central measures. Locally it is possible for the practising veterinarian to influence the project plan through the local public health committee and naturally through direct contact with the farmer.

Regionally two veterinarians specially educated in animal hygiene are appointed by the state to scrutinize all project plans for new building and re-modelling of animal houses. This scrutiny has been compulsory since 1973 for all animal houses except the very small ones. Through this scrutiny - which means that the veterinarian in many cases has to be in contact with the farmer - an effective correction from the point of view of animal health can be made in due time. Through this correction inappropriate plans can be avoided and environmentally

evoked diseases in the herd can be prevented.

Centrally it has been arranged that all drawings and project plans produced by the National Agriculture Board since 1969 have to be scrutinized and approved by an advisory board including two veterinarians. This has led to revision of a great number of drawings and plans which were in use in 1969 and which were suitable from a technical point of view but not from an animal health point of view. Animal environments designed according to these drawings before this revision took place have been shown to cause diseases.

As a result of these new disease-combating measures the state of health in the new animal houses built or re-modelled since 1970 in general seem to be much better than in those built or re-modelled before 1970. The difference becomes even more obvious by a comparison between environments created before 1970 and after 1973. Even if these new veterinary tasks do not reduce the need for veterinary service in the single herd very much, it definitely makes the work in the single herd much more effective and succesful. The number of animal houses with unsuitable animal environments are gradually reduced.

It was possible to apply the model described here in Sweden and it meant a great deal of co-operation between veterinarians and technicians. For obvious reasons it may be necessary for veterinarians in other countries to find other solutions. Irrespective of the methods chosen it is, however, necessary to render the combating of environmentally evoked production diseases more effective. This has to be done by making the practising veterinarian aware of the connections between animal health and animal environment and give him the knowledge necessary for combating environmentally evoked diseases through preventive measures. It is, however, just as important to combat these diseases by means of systematic preventive veterinary measures in an earlier stage than when the herd and the production is already established.

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Summary of the discussion

The discussion centered on the moral and technical aspects. Technically already much is known of the sound burden and the influence on the hormonal system. Effects on the immunity seems possible. Mild electric shocks as reported from New Zealand to cause production losses as high as 20% in

milk parlors, have not been seen here, but studies have been made on the frequency of tit trampling. It is difficult to measure "well being" of production animals. Low mortality and a constant high production are not sufficient to guarantee that animals are well. Maybe it is so in cows and sheep, but certainly not in hens and pigs, with e.g. foot lesions. We do not know yet why these animals, although undoubtedly suffering, will go on producing with high efficiency. The existing economic difficulties for complete combatting a bad environmental situation should be fully understood. Meanwhile we should inform the farmers in order to enable them to make the right decisions.

GLUCOSE METABOLISM IN RUMINANTS

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Summary

Gluconeogenesis is of importance in ruminants since only little glucose usually is absorbed. Glucose is absolutely required by several tissues and its utilization, within limits, is proportional to its concentration in blood. The liver produces about 85% of the glucose and the kidneys the remainder. In fed ruminants, the main glucose precursors are propionate and amino acids. In starvation, glycerol from body fat replaces some of the propionate but most glucose must be formed from amino acids. Alanine and glutamine are the principal amino acids involved in gluconeogenesis and they transport carbon and nitrogen between muscle, liver, gut, and kidneys. Gluconeogenesis is controlled by 1) the supply of precursors and 2) metabolic pathways in liver and kidneys. Both can be involved in the development of hypoglycemia but the control of precursor supply seems to be the critical factor.

Introduction

Most dietary carbohydrates are fermented in the rumen to volatile fatty acids. Thus only small amounts of glucose usually are absorbed and gluconeogenesis becomes of prime importance for ruminant metabolism. Further, the supply of glucose precursors and the organs that synthesize glucose can be limiting factors for the animal's overall productivity and even for its survival. Glucose is needed by at least 5 areas of the body: 1) nervous system, 2) mammary gland, 3) fetuses, 4) turnover and synthesis of fat, and 5) muscle and liver glycogen (Bergman, 1973; Lindsay, 1973; Setchell et al., 1972). While the first three require the most glucose, the needs of all five are variable and depend upon the physiological status of the animal.

This paper will discuss the subject of glucose metabolism on the basis of 4 questions which need answering. 1) How much glucose needs to be produced? 2) What are the organs for glucose production? 3) What are its precursors, especially the amino acids? 4) How is glucose production controlled?

Amounts of glucose produced

Glucose turnover measurements have been used to estimate the amount of glucose produced in the whole body. It is estimated from the rate of dilution of ^{3}H- or ^{14}C-labeled glucose in the general bloodstream (Bergman, 1975; Brockman et al., 1975; Leng, 1970) and represents the flow or entry of new glucose into the blood or, for practical purposes, glucose production by all organs including absorption from the gut. If the blood glucose concentration is constant, the utilization of glucose equals its production and the combined processes are termed a turnover rate.

The turnover of glucose in ruminants is highly variable. It is proportional to the animal's overall metabolic rate and to its feed consumption (Bergman et al., 1973, 1974; Leng, 1970; Lindsay, 1970). In sheep, it is reduced by about one-third during fasting but is always higher during late-pregnancy and highest of all during the peak of lactation. A 50 kg sheep thus can have a glucose turnover of from 70 to 350 g/day. Comparable figures for cattle (Kronfeld et al., 1971; Leng, 1970) are about 500 g/day during the dry period and about 1700 g/day during lactation. Fetal metabolism in sheep uses roughly 40% of the glucose turnover during late-pregnancy (Bergman, 1973; Setchell et al., 1972) and lactose production uses as much as 60-85% in cattle (Kronfeld, 1971), sheep (Bergman et al., 1967) and goats (Annison & Linzell, 1964).

The turnover of glucose also is related to its concentration in plasma. In sheep, it progressively decreases during the development of hypoglycemia meaning that gluconeogenesis fails to keep pace with the animal's requirements (Bergman et al., 1973, 1974). In cattle, during very early stages of hypoglycemic ketosis and if feed intake is not reduced, glucose turnover rates show little change from normal. The low blood glucose thus could be due to an enlarged glucose space and it was suggested that this is associated with an insulin release (Kronfeld et al., 1971). More recent studies (Schwalm & Schultz, 1976, however, have found that blood insulin levels actually are depressed in bovine ketosis. Further, a reduction of feed intake in cattle (Kronfeld et al., 1971) will decrease both the glucose turnover and its concentration as it does in sheep and most cattle during ketosis are either losing weight or in a state of semi-starvation.

Sites of glucose production

In all mammals, glucose can be produced by

the 1) gut (absorption), 2) liver and 3) kidneys. Only recently, however, have direct data been available on ruminants to assess the relative importance of each of these sites.

Absorption from the gut

In cattle and sheep on roughage diets, only insignificant amounts (2-8 g/day) of glucose are absorbed. On 80% concentrate rations, however, some starch can escape fermentation and flow into the intestines for later glucose absorption. Barley and oat diets are largely fermented but maize, if fed in large amounts and finely ground, seems to be able to yield up to 100 g/day of glucose in sheep or 600 g/day in cattle (Hibbitt, 1973; Lindsay, 1970). On most diets, however, glucose absorption surely must be insufficient for the glucose needs of the animal.

Hepatic glucose production

This has been studied in sheep (Bergman et al., 1970, 1974, 1975) by using catheters chronically implanted into the portal, hepatic and renal veins and aorta and measuring venoarterial concentration differences and rates of blood flow. Glucose labeled with ^{14}C or ^{3}H was infused for measuring glucose turnover in the whole body and also for measuring unidirectional rates of glucose utilization and production.

Fig. 1 is a diagram of the flow and metabolism of glucose as obtained in fed sheep. No absorption of glucose was detected and hepatic production accounted for 4.5 g/hr or about 85% of the total glucose turnover. Glucose utilization by both liver and portal-drained viscera was small as compared with other tissues (e.g. brain, muscle).

Renal glucose production

The 0.7 g/hr (15%) of glucose produced by peripheral tissues (Fig. 1) was found to be mostly due to the kidneys. During fasting, total glucose production always decreases but during either fasting or feeding the sum of that produced by both liver and kidneys was 94-107% of the total glucose turnover.

Precursors of glucose

Amino acids, propionate, glycerol and lactate are the only significant precursors for gluconeogenesis in the ruminant (Bergman, 1973; Exton, 1972) and all of these, except glycerol, funnel into oxaloacetate before being converted to glucose or glycogen.

Amino acids as glucose precursors

Gluconeogenesis from amino acids can vary widely depending upon the nutrition and physiologic state of the animal. Two approaches recently have been used to estimate their contribution to glucose: 1) the transfer of ^{14}C from labeled amino acids and 2) the net hepatic and renal uptakes of amino acids from plasma as measured by venoarterial concentration differences and rates of blood flow. The first gives a minimal estimate for the whole body since some ^{14}C can cross over to the TCA cycle from oxaloacetate and also since the ^{14}C specific activity within the cells may not be fully equilibrated with that in plasma (Lindsay, 1970; Wolff & Bergman, 1972). Conversely, the second approach gives a maximal estimate since liver uses amino acids for synthesis of protein as well as glucose.

Using ^{14}C-labeled amino acids in fed cattle and sheep (Brockman et al., 1975; Egan & Black, 1968; Heitmann et al., 1973, 1976; Reilly & Ford, 1971; Wolff & Bergman, 1972), 10 to 25% of the glucose was calculated to be formed from amino acids with alanine and glutamine-glutamate being the largest contributors. On the basis of net hepatic removal in fed sheep (Wolff et al., 1972), however, it was found that amino acids can contribute up to 32% of the glucose; renal removal (Bergman, 1973) could yield another 2 to 3%. Alanine and glutamine again were the greatest contributors accounting for over 40% of the hepatic removal of all amino acids. Glycine and serine were next in order of removal by accounting for 25%. Overall results thus show that fed ruminants probably derive a minimum of about 15% and a maximum of about

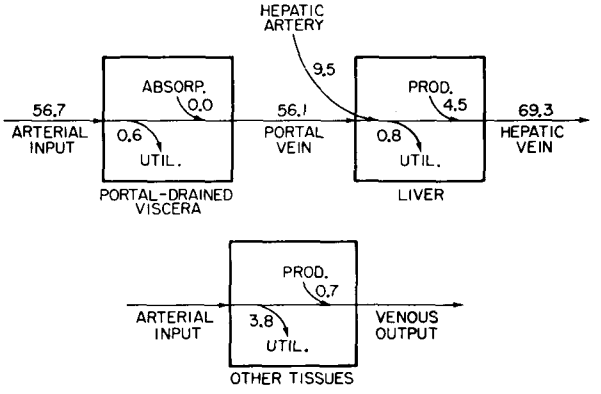

Fig. 1. Flow diagram of glucose (g/hr) in tissues of 10 nonpregnant sheep fed hay and grain (50:50) at hourly intervals (800 g/day). Mean glucose turnover was 5.2 g/hr (from Bergman et al., 1974; 1975).

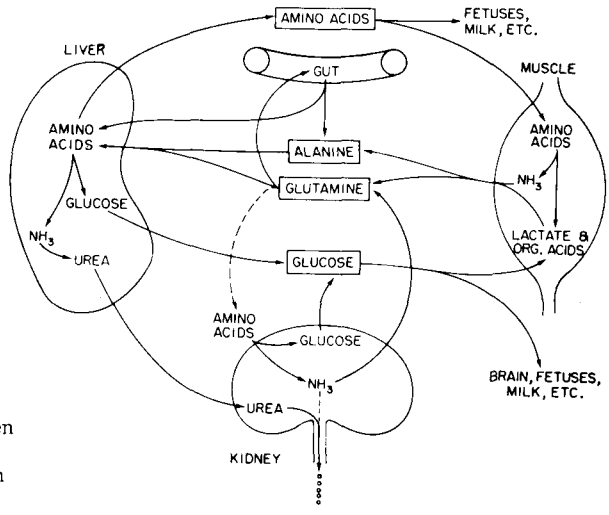

Fig. 2. Relationship of amino acids to gluconeogenesis in fed ruminants. Alanine and glutamine always are released by muscle and removed by liver. Dashed line indicates acidosis or starvation (from Bergman et al., 1973).

35% of their glucose from amino acids.

Alanine and glutamine cycles thus have been proposed (Ballard et al., 1976; Bergman, 1973) as a means of linking amino acids with gluconeogenesis (Fig. 2). They seem to be major vehicles for both nitrogen and carbon transport from muscle to liver although their cycles are modified by both gut and kidneys. While animals at maintenance have a daily amino acid requirement, most amino acids eventually are converted to glucose and urea.

Propionate, glycerol and lactate as glucose precursors

In fed ruminants, propionate probably is the largest glucose precursor (Bergman, 1973; Hibbitt, 1973; Leng, 1970). On hay diets it can supply up to 40% of the glucose and even more if grain is fed. Glycerol and lactate each have been reported to contribute 5 to 15% (Annison et al., 1963; Bergman, 1973; Brockman et al., 1975). After 2 to 3 days of starvation, however, propionate absorption drops to negligible amounts and the animal must shift to glycerol and amino acids for its glucose precursors. Under these conditions, glycerol has been shown to contribute 23 to 45% of the glucose and amino acids must contribute nearly all of the remainder.

Control of glucose production

Control of precursor supply

Gluconeogenesis in ruminants increases after feeding and decreases during starvation simply due to the availability of precursors, especially propionate and amino acids (Katz & Bergman, 1969). Adequately fed ruminants should not be short of precursors but if not eating, or if large amounts of glucose are needed for high productivity, precursors must be mobilized from body stores of protein and fat.

The hormonal control of precursor supply is complex and involves both short-term and long-term actions. Nearly all hormones can be involved especially insulin, glucagon, gastrointestinal, adrenal and pituitary hormones (Bassett, 1975). Insulin (Brockman et al., 1975) appears directly to affect muscle and adipose tissue to decrease precursor supply; it has energy storage or anabolic actions. Glucagon, however, is a catabolic hormone and increases net amino acid and glycerol outflow from muscle and fat. In addition, it directly stimulates the liver to take up amino acids for increased gluconeogenesis. The ratio of insulin to glucagon in the blood thus seems to be of great importance in regulation of precursor supply (Bassett, 1975). Adrenal steroids are used clinically to increase blood glucose concentrations. Their actions seem to be 2-fold since they increase amino acid output from muscle for increased glucose production (Heitmann & Bergman, 1976) but they also decrease milk production for glucose conservation.

Control of gluconeogenesis in liver and kidneys

The uptake and metabolism of glucose precursors are believed to be controlled by key enzymes (Baird et al., 1968; Bergman, 1973; Hibbitt, 1973); 1) glucose-6-phosphatase, 2) fructose-1,6-diphosphatase, 3) propionyl-CoA carboxylase, 4) pyruvate carboxylase for formation of oxaloacetate from pyruvate and 5) PEP carboxykinase for conversion of oxaloacetate to P-enolpyruvate. The first two are overall controls for the flow of metabolites to glucose but the last three are more specific. Pyruvate carboxylase may be of particular importance for ruminants since it is stimulated by propionyl-CoA and butyryl-CoA which increase after feeding. It also is stimulated by glucagon. Thus, an increased liver uptake of lactate, alanine and glutamine for gluconeogenesis will occur (Brockman et al., 1975).

Oxaloacetate may be a link between gluconeogenesis and fat metabolism in liver since

it is a central intermediate for glucose synthesis as well as for oxidation of acetyl-CoA. If oxaloacetate is deficient, insufficient glucose will be formed and less fatty acids oxidized to CO_2. Hypoglycemia thus can occur and liver fat catabolism diverted to ketogenesis. Depressed oxaloacetate levels have been reported in livers of ketotic cows (Baird et al., 1968; Hibbitt, 1973) and the most plausible cause is a lack of precursors (propionate and amino acids) in relation to the required high rate of gluconeogenesis.

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Summary of the discussion

Any glucose formed in the kidneys will be effectively removed by the liver and therefore the importance for the peripheral metabolism is small. Specific amino acids are taken up by the liver, in proportion to their concentration in the blood. The greater glucose turnover in lactating sheep will result in a marked mobilization of body fat during the first month, but even so the production level remains well below that in cows, so that acetonemia is not likely to occur during lactation, in contrast with pregnant ewes carrying twins or triplets. It was remarked (Prins) that in these long term studies one would expect underestimation of the turnover rate caused by recycling of the label, for instance by way of the lactate. Nevertheless the ^{3}H results are nearly identical with the ^{14}C ones. Although glucose is not absorbed in any important quantity from the G.I. tract it may be assumed that lactate interconversion in the rumen epithelium may save some for gluconeogenesis.

PREPARTAL FEEDING AND THE LEVEL OF FEEDING AT THE PEAK OF MILK PRODUCTION

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Summary

During the dry period, the nutrient intake of cows normally exceeds the requirements for the development of the fetus, for the expansion of the uterus and for the preparation of the mammary gland for the following lactation. A surplus of energy is mainly stored in the form of body fat, and only small amounts as glycogen in the muscles or liver.

At the beginning of lactation, body fat can in part be directly utilized for the synthesis of milk fat.

As the milk yield increases and there is a more intensive stress in energy metabolism, the glucose level of the blood falls. Simultaneously a greater concentration of ketone bodies appears, because the breakdown of body fat is inhibited by a lack of oxalacetate, used for the synthesis of lactose. A high level of ketone bodies in the blood just at the time of the peak in milk production reduces the feed intake, and the deficit in energy supply is exacerbated.

Consequently, energy storage in the dry period in form of body fat prevents a nutrient supply appropriate to the milk yield at the peak of lactation.

Introduction

The standard of performance in dairy cows could be raised by selection, and by an improved nutrient supply. At the same time the physiological stress of the total organism is also raised, causing more metabolic diseases and disturbances to fertility. Performance therefore not only involves mechanically measurable parameters, such as milk yield and milk fat content, but has especially to take into account physiological reactions to exogenous influences and endogenous processes. The most important exogenous factor is the nutrient supply. There are very clear theoretical conceptions concerning the nutrient requirements of the dairy cow. The requirement for the production of one unit of milk with various fat contents is well known. But it is also common knowledge that just at the beginning of lactation, at the time of the highest nutrient requirement of the dairy cow, it is hardly possible to reach the nutrient intake necessary for milk production. Moreover, this is frequently the period for metabolic diseases and disorders in fertility.

The origin for many of these negative reactions of the organism could probably be found in pregnancy, mainly in the dry period. For this period there exists no norm for the nutrient requirement of the dairy cow. Under practical conditions it is recommended to feed nutrients for 10 - 12 kg of FCM above maintenance. Because the requirement for fetus, uterus and mammary gland is much lower, a surplus in energy must be stored, mainly as body fat.

At the beginning of lactation, prolactin influences the mobilization of these reserves, which under special circumstances in the metabolism of energy can be utilized for the synthesis of milk. These changes from positive to negative nutrient balance is proved in several metabolism experiments with dairy cows by FORBES (1922, 1935), ELLENBERGER (1931, 1932, 1950), PIATKOWSKI (1962, 1964) and OSLAGE (1966, 1970), with sows by LENKEIT (1955, 1956) and with goats by KALAISSAKIS (1958, 1959).

These studies show that an increased storage during late pregnancy is necessary for compensation of the nutritional deficit at the beginning of lactation.

The goal of a special experiment with dairy cows, made in our Institute, was to investigate the influence of storage and mobilization on milk production, energy metabolism, and fertility.

Results and discussion

60 dairy cows of the German Black and White breed were fed at widely differing nutrient levels in the

dry period and at the beginning of lactation. The experimental design is shown in Table 1.

Table 1: Experimental Design
(Ketosis Experiment 72/73)

Exp. group	n	Energy supply during last 10 weeks a.p.
I	30	maintenance + 16 kg FCM
II	30	maintenance + 2 kg FCM

Exp. group	n	Energy supply 1.-10. week p.p.
I a	15	norm
I b	15	norm - 30%
II a	15	norm
II b	15	norm + 30%

a)Variation in body weight.
Cows of Group I had a mean weight gain of 86 kg during the dry period. For Group II, this was 23 kg, which is mainly due to the growth of the fetus, indicating that these cows did not store nutriants. The weights of the calves were only slightly different, with a mean of 40 kg for Group I and 38 kg for group II. Similar results are mentioned by GARDNER (1969). The curve of body weight in the first 10 weeks of lactation is directly effected by the nutrient supply in the dry period and in lactation. The higher the storage, the more intensive and the more persistent is the loss of body weight. It amounted to 47 kg for Group Ia, 77 kg for Group Ib, only 18 kg for Group IIa, while Group IIb had an average weight gain of 6 kg.

b)Nutrient conversion
The utilization of nutrients for milk synthesis follows the curve of body weight. The cows of Group Ib show a strongly negative balance during the whole experiment; animals of Group Ia and IIa reach a positive balance between the 6th and 7th week of lactation; Group IIb in the 2nd week.

c)Milk yield
The milk yield in FCM is distinctly different between the 4 groups. Animals with high nutrient supply in the dry period have a higher milk production of about 2 kg FCM/day. This was mainly due to the higher milk fat content. The composition of milk fat shows that body fat had been directly converted into milk fat. This is especially evident in cows with a high reserve from the dry period and a low energy intake in early lactation, which had a body weight loss of about 77 kg. This demonstrates that a high milk fat content can be obtained not only by rations rich in cellulose, but also by metabolized body fat produced during pregnancy. The economy of this method is very doubtful. Even if these results seem to encourage the deposition of body fat concerning milk yield and milk fat the amount of physiological stress should be taken into consideration. Here, relationships between nutrient supply in the dry period and disturbances in the energy metabolism at the beginning of lactation become evident.

d)Glucose in blood
Blood glucose during the dry period was not dependent on the nutrient supply. The concentration was between 45 - 50 mg/100 ml with a distinct increase just before parturition. From the beginning of lactation up to the 4th week the glucose level drops markedly in all groups. This occurred in animals with a high nutrient supply during the dry period much more than in the others. Thus when body fat is mobilized, the energy needed for this process, coupled with a high lactose output, creates an energy deficiency. When milk yields inverse to the peak of lactation, the breakdown of body fat cannot be reached exoenergetically. This process is stopped at the stage of ketone bodies. The production of Ketone bodies is inversely related to the glucose level.

e)Ketone bodies
The ketone bodies acetone, acetoacetate and 3-hydroxybutyrate were analysed separately, but here they are shown as a total. The highest values are observed at the time of lowest glucose concentration, i.e. at the peak of lactation. Animals with a high loss of body weight

show the highest concentration of ketone bodies, more than 30 mg/ 100 ml. As already mentioned, the milk fat content was also extremely high in these animals, due to a direct transition from body fat to milk fat, particularly if the cows are forced to mobilize body substance because of low supply in early lactation. The accumulation of ketone bodies leads to a decrease in feed intake and exacerbates the imbalance between energy required and energy available. Thus a drop of milk yield will follow. This mobilization of body mass stored during pregnancy, frequently causes a clinical ketosis.

The possible relationships between feeding metabolism and performance have been discussed by KRONFELD (1976) and DREPPER (1976) in a detailed literature study.

Our results demonstrate that in animals with high body loss in clinical ketosis, it is not possible to balance the energy metabolism by normal application of energy rich substances such as glucose or propionate. Shortly after treatment, ketosis may appear again. Under practical conditions the observation that it is very difficult to stabilize energy metabolism is also often made.

Investigations of JAZBEC (1967) with a large number of dairy cows demonstrate that ketosis mainly occurs during the first 5 weeks of lactation, which corresponds with the maximum mobilization of body mass.

f) Fertility

The most important features of reproductive activity are negatively influenced by a surplus of energy in the dry period. Involution of the uterus is completed in the first 4 weeks of lactation in 43% of the high fed cows v. 83% in the low fed animals. The comparable results for endometritis are 71% v. 27%; frequency of infections of genital tract 55% v. 23%; follicular cysts, 45% v. 19%; and paralysis uteri 26% v. 6% (LOTTHAMMER, 1974).

In summary, it is evident that the preparation of dairy cows for the next lactation, as is common under practical farming conditions, is neither to be recommended from the economical point of view, nor from physiological aspects. A high fat deposition in the dry period cannot in general provide for the higher requirements in early lactation. An energy storage in the form of body fat can surely be utilized for an increase of milk fat. However, there will be a simultaneous increase in ketone bodies resulting from a heavy stress of energy metabolism, and causing a decline in feed intake. Thus neither by amino acids nor by carbohydrates can enough precursors for the synthesis of lactose be made available. In this cycle oxaloacetate has a key position. This situation can only partly be compensated for by feeding concentrates at the peak of lactation, and it is still difficult to save the milk fat content.

A nutrient intake, supplying the requirements for performance and which takes into account the special digestion physiology of the ruminant in early lactation, is only feasible, if the deposition of body fat in the dry period is as low as possible. The feeding level at the peak of lactation is directly dependent on the intensity of the nutrient supply during the dry period.

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Summary of the discussion

Addition of fats to the ration in early lactation in order to prevent breakdown of body fat seems interesting, but it cannot be expected to solve the problem of ketosis. The fats, of course, should be protected in order to overcome the fermentation in the rumen. The loss of body mass during the early period of milk production is mainly in the form of body fat; the conversion to milk fat occurs on a large scale by an "easy" pathway of metabolism. Some discussion arose as to the duration of the dry period as a factor determining the relation of "fit and fat": for the following lactation the animals should be made fit, and not too fat. One cannot generalize on the advisability of "steaming up"; it is said to be advisable for cows having a very high milk production, at least in so far as the reserves consist of protein in addition to fat. Some cows on the high concentrate diets showed higher GOT levels than usual; autopsy confirmed that liver damage existed. A high protein intake during the dry period caused higher GOT levels in (early) lactation.

PLASMA GLUCOSE AND NON-ESTERIFIED FATTY ACIDS IN RELATION TO DIETARY ENERGY INTAKE IN THE DAIRY COW

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Summary

The effects were studied of 6 different energy intake patterns during the first 20 weeks post partum on milk yields, body weights and plasma glucose and non esterified fatty acid (NEFA) concentrations of groups of high yielding Friesian dairy cows.

The different energy levels were reflected in body weight changes and/or milk yields but were not directly related to the plasma component levels. The direction of the partition towards milk production or body condition varied between feeding groups and between individuals within a group.

The group mean patterns for plasma glucose and NEFA were similar for all groups and changes in the energy intake levels at 10 weeks produced responces in body weight or milk yield rather than in the plasma component levels.

Plasma glucose and NEFA levels were significantly correlated with deviations from energy balance based on the rate of body weight change but the calculated values for zero body weight change showed considerable variation. This may be due to the error inherent in using body weight change as a standard of energy balance as well as to factors other than energy balance which affect the plasma component levels.

Introduction

The difficulty of estimating with reasonable accuracy the nutrient intake of dairy cows under commercial farm conditions has encouraged attempts to identify components of blood which can be used as indices of nutrient adequacy. The use of selected groups of cows to assess herd nutritional and metabolic status is well established and is an integral part of eg. the Compton Metabolic Profile Test (Payne et al 1970) and herd health monitoring techniques (eg. Blowey 1972).

Whilst it is consistent with economical milk production that a high yielding dairy cow during early lactation should undergo a period of energy deficit, knowledge of the extent and duration of this deviation from the balanced state is useful in achieving the optimal feeding pattern.

As an alternative to serial body weight determinations to assess the adequacy of energy intake during lactation, use has been made of blood component concentrations eg. glucose, NEFA, ketone bodies, but their practical value in this context has been questioned on the basis of the influence of homeostatic mechanisms (eg. Rook & Line,1961) and the variability in the relationship (eg. Erfle et al 1974, Fisher et al 1975).

The experiment described was intended to further evaluate the use of plasma glucose and NEFA, particularly on a group basis as indices of (a) the level of energy intake (b) the deviation from energy balance.

Materials and Methods

48 cows were randomised into 6 groups equivalent according to previous milk yield, age (from 2nd to 6th lactation) and body weight. All groups were fed a similar "steaming up" ration and then each group was allocated to one of 6 feeding treatments. These consisted of 6 permutations of 3 constant energy levels (E_1, E_2 and E_3) for the first 10 weeks after calving and 2 constant levels (E_1 and E_2) for a further 8 weeks, after a 2 week change over period.

The feeding levels E_1, E_2 and E_3 provided approximately 125, 150 and 170 megajoules per day respectively according to Ministry of Agriculture, Fisheries and Food (1976). The concentration of energy in the 3 diets was similar and protein intake was not a limiting factor. The diets consisted of concentrates and silage.

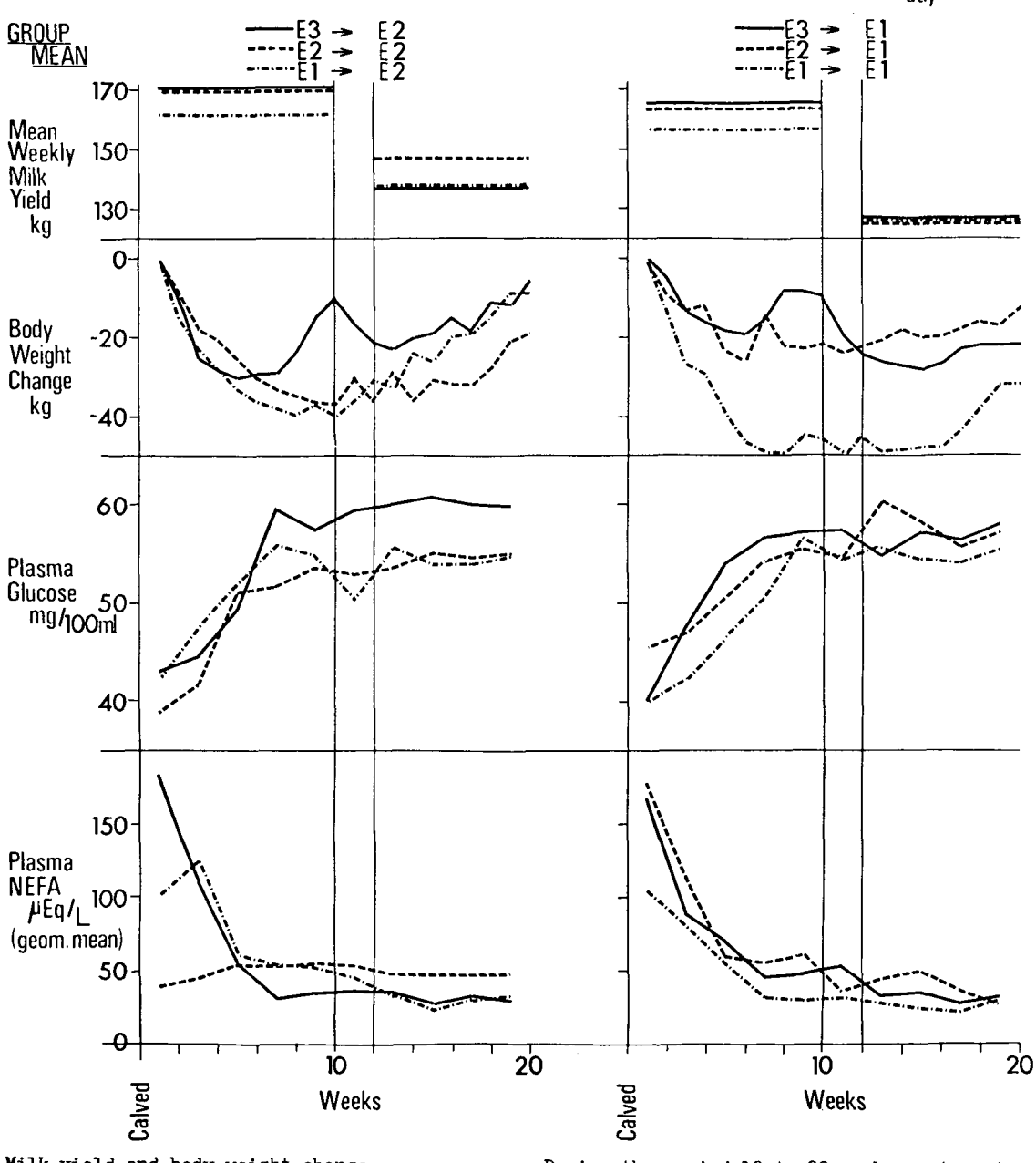
The daily routine was:-

0545-0615	hrs	Morning milking, fed 0.5 kg concentrates
0700-1000	"	Blood sampling (every 2 weeks)
1000-1100	"	Exercise
1500-1530	"	Afternoon milking, fed 0.5 kg concentrates
1700-	"	Fed silage and concentrates

By 2-4 weeks after calving the cows consumed all the feed presented.

Weekly milk yields, milk composition and body weights were recorded and every 2 weeks coccygeal blood samples were taken.

Fig. 1. Energy intake levels in relation to group mean milk yields, body weight changes and blood component concentrations.



Milk yield and body weight change

During the first 6 weeks of lactation the mean body weight of all groups declined as energy requirements for milk yields exceeded intakes but the fall was less marked and reversed sooner in the group on the higher energy levels.

During the period 12 to 20 weeks post partum, energy intake was partitioned more towards restoration of body condition than to increasing milk yield but there was considerable individual and group variation in the extent to which this occurred.

Blood component levels

The pattern of serial blood glucose and NEFA measurements for all groups was similar over the 20 weeks studied. The mean plasma glucose concentration of all groups was low immediately after calving and attained values of 50-60 mg mg/100 ml by 6-8 weeks, after which the levels remained relatively constant despite changes in energy intakes at 10 to 12 weeks post partum.

Mean plasma NEFA values tended to be highest in early lactation becoming relatively constant at below 50 µEq/L by 8 weeks.

Again the change at 10 weeks produced no obvious response in NEFA levels.

The group mean plasma component concentrations did not therefore directly reflect the energy intake levels.

Relationship of energy balance to blood component concentrations and body condition

This relationship was studied by means of regression analyses of rate of change of body weight at each blood sampling on plasma glucose and log NEFA concentration for individual cows.

The mean weekly body weight change was estimated over a 2 week period from a quadratic curve if it appeared to be a good fit. Where it was apparent that there were major deviations from such a curve the change was determined from moving averages calculated over 3 week periods. Graphs of serial body weight for 8 cows were considered too erratic to make satisfactory estimations of rate of body weight change and the data from these cows was not included in the regression analysis.

Although rate of body weight change was highly significantly correlated with plasma glucose ($p<0.001$) and log plasma NEFA ($p<0.001$), when the plasma component concentrations for zero body weight change were calculated, the 95% tolerance limits were large (Table 1) thus limiting their usefulness as indices of energy balance. However the individual variation may partly be due to the error in the standard used for energy balance (i.e. rate of body weight change) as well as to factors other than energy status which influence the plasma component concentrations.

Table 1. Calculated mean plasma component concentrations at zero body weight change

	mean ± standard error	95% tolerance limits
plasma glucose (mg/100 ml)	53.95 ± 0.564	47.0-60.9
log plasma NEFA	1.630 ± 0.0195	1.389-1.870
geometric mean plasma NEFA (µEq/L)	42.62	24.5-74.2

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Summary of the discussion

This work was part of another experiment, and consequently the energy inputs to the various groups were fixed. The results from one cow that suffered from a persistant ketosis were excluded from the calculations. Addition of fat to the rations is more ketogenic than glucogenic. In the early period of lactation weight loss seems to be unavoidable, later on glucose can cope with the loss of body weight.

GLUCONEOGENESIS FROM AMINO ACIDS IN LACTATING COWS

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Introduction

In ruminants most of the carbohydrates in the food are broken down into volatile fatty acids. Of these,only propionic acid can serve as a precursor for glucose. If insufficient sugars can be absorbed from the ration to meet the glucose requirement, the body synthesizes glucose from other compounds such as propionic acid and glucogenic amino acids.

The need to use amino acids for the synthesis of glucose may lead to an increase in protein requirement. Some information on the essentiality of the use of amino acids for glucose synthesis may be gained from a comparison of the glucose supply and glucose requirement.

Glucose supply from absorbed glucose and propionic acid

As an example the supply will be calculated for a cow of 500 kg body weight and a milkproduction of 10 kg. For 20 and 30 kg milk only some figures will be given. The ration consists of 7 kg hay and concentrates according to requirement for the assumed production or about 5, 9.5 and 14 kg resp.

1 Absorbed glucose. It is assumed that the α-glucose-polymer content of the concentrates is 30 % and that the amount of absorbed glucose is about 10% of the starch ingested. In fact both these figures can be much higher or lower. From the assumptions it can be calculated that 150 g, 280 g or 410 g α-glucose-polymers reach the duodenum, partly as starch that escaped fermentation, partly as microbial polysaccharides.

2 Maximum glucose synthesis from propionic acid. For the calculation the next assumptions are made:

- metabolizable energy requirement, M_E, for maintenance is 115 kcal and for production 1250 kcal per kg milk
- ruminal heat production is 10% of M_E
- energy in protein and fat is 20% of M_E
- energy in α-glucose is 4.2 kcal per g
- molar and weight ratios of acetic, propionic and butyric acid are 60/24/16 and 53/26/21 resp. (ratios may be different for different production levels)
- energy content of the fatty acid mixture is 4.4 kcal/g
- 148 g propionic acid (2 mols) are converted in 180 g glucose (1 mol) if the conversion is maximal.

The calculation for a cow producing 10 kg milk is:

energy requirement, M_E		
maintenance	12.0	Mcal
production	+ 12.5	
M_E requirement	24.5	
ruminal heat	- 2.45	
energy in protein/fat	- 4.90	
energy in α-glucose	- 0.65	
energy in fatty acids	16.50	Mcal
amount of fatty acids	3750	g
amount of propionic acid	975	g
maximum glucose synthesis	1185	g

An equal calculation for the 20 and 30 kg milk producing cow results in a maximum glucose synthesis from propionic acid of 1775 g and 2365 g respectively.

A quantitative conversion of propionate in glucose will never be reached. In literature figures can be found ranging from 25-70 percent. Most figures, however, refer to adult, non pregnant, non lactating sheep with an ample supply of protein in the ration. The surplus of amino acids can be used for glucose synthesis in the same way as propionic acid as both amino acids and propionic acid are converted into oxaloacetate on their way to glucose. In most experiments from the literature, therefore, glucose requirement is low and the surplus of amino acids results in a high conversion of amino acids into glucose and a rather low conversion of propionate.

As here the question is whether amino acids need to be used,more than whether they are used,figures will be calculated for both 100 and 60% conversion.

Glucose supply:

conversion, %	100			60		
milkprod., kg	10	20	30	10	20	30
from propionate g	1185	1775	2365	710	1065	1420
from α-glucose g	165	310	450	165	310	450
supply, g	1350	2085	2815	875	1375	1870

Glucose requirement

The glucose requirement for maintenance was calculated from data available from the literature (Boekholt, 1976. Ph.D. Thesis, in press).

Non pregnant, non lactating sheep fasting for at least 24 h have on average a glucose entry rate of 3.30 mg/min/kg$^{\frac{3}{4}}$. This agrees well with two figures for cows. For a 500 kg weighing cow the glucose requirement will be 500 g.

For the synthesis of 1 mol lactose (342 g) the animal needs 2 mols glucose (360 g). For 1 kg milk with about 50 g glucose are used for the synthesis of lactose (4.8%). About 12% of the milkfat consists of glycerol. So about 5 g glucose or glucose precursors are utilized in the synthesis of milkfat come from the pentosephosphate cycle for 1 kg fat 730 g glucose are needed, or for 1 kg milk, 4% fat, about 30 g glucose. Bauman et al. (1972-1973) assume that about 50% of the NADPH can be synthesized in the cytoplasma with the help of NADP-isocitrate dehydrogenase. According to this it is assumed that 15 g glucose is utilized for the generation of NADPH from glucose. In total the animal needs 70 g glucose for the production of 1 kg milk.

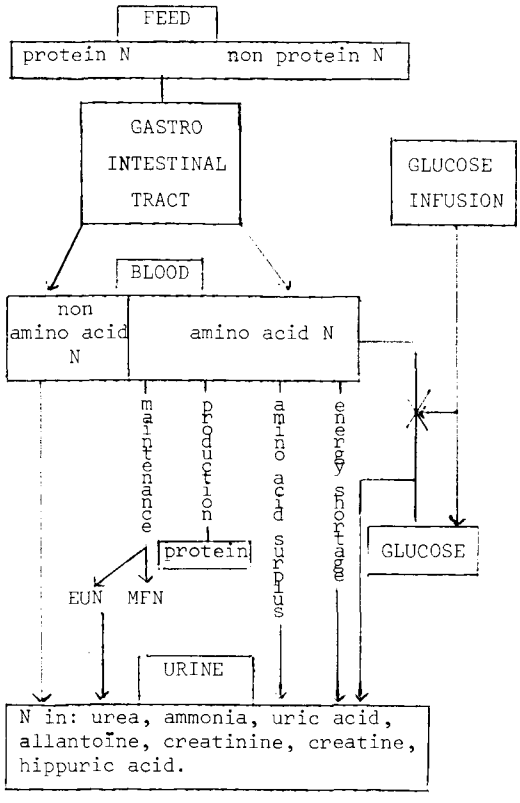
Glucose requirement:

milkproduction, kg	10	20	30
maintenance, g	500	500	500
milkproduction, g	700	1400	2100
	1200	1900	2600

The comparison of glucose supply and requirement does not clearly answer the question whether the use of other compounds than glucose and propionic acid is essential to meet the glucose requirement. Especially the estimation of the glucose supply is uncertain.

Most experiments on the use of amino acids for glucose synthesis are carried out with sheep. The experiments clearly demonstrate the synthesis of glucose from amino acids. The literature often assumes that amino acids are an important source of glucose. However, the synthesis of glucose from amino acids as a result of a surplus of amino acids is not distinguished from that as a result of shortage of glucose synthesized from other precursors. Most experiments concern the former situation. That is why these experiments give no information whether amino acids are indeed necessary to provide in a glucose shortage.

Gluconeogenesis from amino acids was studied with full-grown lactating cows. The intake and excretion of N was measured under normal circumstances and during administration of glucose through a fistula into the duodenum or by infusion into the blood. The Figure schematizes the intake and excretion of N. If amino acids are used for glucose synthesis, the N of the amino acids is excreted in the urine as urea. If the synthesis of glucose from amino acids is decreased by external administration of glucose into the blood and if the freed amino acids are utilized for protein synthesis, the amount of urinary N decreases This decrease is a measure of gluconeogenesi from amino acids.



All in all 8 comparisons were made of cows with a glucose infusion, ranging from 200-900 g/day, and without. The preliminary period for the blank and infusion experiments lasted for 7-14 days, and the experimental periods were 7-10 days each. In one experimen glucose or water was alternately infused for 4 to 5 days each.

During glucose infusion no glucose was observed in urine. The glucose concentration of the blood plasma was normal, even shortly after the infusion started. On the whole the milkproduction increased during the first 2 or 3 days of the infusion. Afterwards the production decreased to the normal level. Hence, no increase in production was observed during the experimental period. The productio increase during the first couple of days was mostly accompanied with a decrease of the protein content. The administration of glucose more often than not resulted in a decrease of the fat content. The lactose content was less variable than the contents of protein and fat and tended to decrease during the glucose infusion.

Both in the balance experiments with a protein poor ration (5-8) and in the

experiments with a normal protein supply (1-4) no differences showing the presence of gluconeogenesis from amino acids could be observed between the blank and infusion periods. In the experiment with alternate infusion of glucose and water, however, a distinct influence of the glucose was observed. In this experiment only 75% of the decrease in the excretion of urinary N was caused by a decreased excretion of urea and ammonia. At a production rate of less than 25 kg of milk no evidence was found for the assumption that amino acids are needed to meet the glucose requirement. At a higher production rate, however, the use of amino acids for glucose synthesis may occur. At an ample protein supply glucogenic amino acids may be used for glucose synthesis. This does not mean, however, that amino acids are needed for this process.

Summary of the discussion

There should be no misunderstanding as to the need for glucose in milk fat production; there is of course no conversion into milk fat, but the energy will be used to couple acetate molecules through NADPH-formation. The calculation of 60% efficiency of propionate conversion into glucose holds for cows in a marginal protein supply. The differentiation of the levels of amino acids needed for maintenance and gluconeogenesis seems to be justified. In maintenance a large proportion of the amino acids will be needed for metabolic fecal protein production and for intermediary metabolism. Glucose infusions increase the milk yield to such an extent that a much greater total quantity of protein will be secreted despite the depressed protein percentage in milk. The latter seems to be less influenced by the amino acid supply and more so by the total production level.

METABOLIC DISORDERS IN HIGH-YIELD DAIRY COWS PRIOR TO AND POST PARTURITION

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The frequency of metabolic disorders has increased, especially in high performance stock such as dairy cows (Samson, 1973). In these animals, the critical period surrounds parturition with a marked susceptibility to clinical disorders just after calving (Coppock et al., 1974). The major factor of this increased incidence of metabolic disorders is intensified production e.g. in Israel the average milk yield for a 305-day lactation period in 1975 reached 7 191 kg with 228.6 kg of butterfat (Mayer, 1976). Such a performance requires a perfect nutritional balance, especially with regard to level of energy intake and mineral constituents of the diet. Failure to maintain this balance will result in changes in the milk yield and may or may not be accompanied by the occurrence of clinical signs (Hoden & Journet, 1971).

Metabolic diseases and energy balance

(i) Steatosis

In most cases, liver steatosis is subsequent to an increase in the blood level of free fatty acids by lipolysis from the fat reserves. A major factor of the mobilization of fat reserves from the lipocytes is a 'state of fasting', be it relative or complete. Complete starvation occurs in cattle during long distance transportation, and especially in the case of females at the end of gestation, the occurrence of liver steatosis may be considered as a common phenomenon (Glawischnig et al., 1972 ; Venturoli et al, 1974).

A relative state of starvation is experienced by dairy cattle due to loss of appetite at the beginning of their lactation period. Diminution of the level of food intake during the last weeks of pregnancy is well known. The amount of food intake then increases slowly after calving and reaches a peak between the 4th and 5th months of lactation. Several factors increase the risk of liver steatosis at the beginning of lactation. Among them, the ability of high-yielding cows to utilize their body reserves more effectively than cows with a lower production capacity (Hoden & Journet, 1971) and an unbalanced feeding programme. Mayer divides lactation into three three-monthly periods. During the 1st, the animal makes use of its body reserves ; during the 2nd the intake-output ratio becomes more balanced ; the 3rd period permits the reconstitution of body reserves. Thus, for an animal reaching the drying-off period in good condition, overfeeding with high energy feedstuffs such as corn-silage which leads to excessive fattening at parturition is to be avoided (Lamothe et al., 1971).

A lack of lipotrophic factors represents another feature of steatosis. The release of triglycerides into the blood by liver cells requires a supply of low molecular weight lipoproteins. These are formed by protein linked to cholesterol esters and more especially phospholipids specificity of the complex. Methionine provides the labile CH_3^- groups needed for the synthesis of choline which is a precursor of the lecithins released into the efferent hepatic blood by way of the 'very low density lipoproteins'. This explains why the lipid fraction in blood is increased in cows fed with methionine analogues (Patton et al., 1970) and why animals receiving D-L-methionine exhibit a larger amount of butterfat in their milk (Remond et al., 1971). For the same reason there is a significant difference between the liver lipid and phospholipid level of animals treated with an anti-lipotrophic substance and that of animals receiving choline, m-inositol, folic acid and vitamin B_{12} (Smith et al., 1974).

It has also been shown that dairy cattle kept on a solid diet or deprived of food and water (Manns, 1972 ; Cakala & Bieniek, 1975) may develop within 24-48 hours hepatic lesions characterized by glycogenolysis, high lipid levels and biochemical signs (hypoglycemia, high blood level of ketone bodies, free fatty acids) accompanied by functional disorders of the liver (delayed BSP elimination, increase in the blood level of bilirubin). Various forms of liver insufficiency including distomatosis or the result of its treatment, mycotoxicoses, cirrhosis associated with diets based on corn-silage, probably play a major role in the development of liver failure at an alarming rapidity.

(ii) Ketosis

The most specific biochemical characteristics of ketosis are a high blood level of ketone bodies, free fatty acids, acetate, hypoglycemia, and for the liver glycogenolysis and lipid accumulation. In the light of such observations, a distinction between

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ketosis and steatosis appears somewhat arbitrary. However, some clinical observations suggest physiopathological differences. For example, Kronfeld (1972) noted in a group of 10 cows fed identically and with the same milk production, the development of ketosis in 3 of the cows without any prior change in food intake. Baird et al. (1974) in a comparison between starvation ketosis and spontaneous ketosis found in both cases similar biochemical disturbances (diminished hepatic oxaloacetate and increased production of acetyl CoA), however the ability to re-establish the energy balance of milk production only occurred in the case of starvation ketosis. According to the same authors, dairy cows of high genetic potential which have depleted their glucidic reserves and used fat reserves during the phase of negative energy balance never develop ketosis.

In spontaneous ketosis, production of ketone may be disturbed at three levels : stomach, liver or udder.

- In the rumen wall ketogenesis becomes excessive in association with certain types of feedstuffs : silage with normal high levels of butyric acid, or high levels resulting from the conversion of lactate, or young grass or cow cakes which are rich in saturated C_5-C_{19} fatty acids (Dirksen, 1974).
- In the liver, an increased ketogenesis seems to be related to high milk-yield. During lactation, the udder secretes large quantities of glucose (1.24 kg per day for 20 kg of milk), from which the milk lactose is formed. In heavy milkers, if the energy intake is insufficient, or if the ratio of glucogenic (or anti-ketogenic) to lipogenic substances is too high or if the protein level in the diet is too high (Kronfeld, 1972 ; Baird et al., 1974), the mobilization of peripheral fat results in the release of fatty acids. These may be stored in the liver, oxidized by way of Krebs cycle, or converted to ketone bodies. It is not yet known why this 3rd pathway is predominant in ketotic animals (Butler, 1974). Relation with the diminished availability of oxaloacetate is obvious although the origin of the diminution in oxaloacetate is not yet understood. Excessive utilization during glucose synthesis ? Disturbance in synthesis of oxaloacetate by the absence of precursors like propionate, cobalt, vitamin B_{12} ? The precise role of hormonal secretions in ketogenesis is also unclear. There is a tendency for ketotic cows to suffer from hyper- and then hypoinsulinemia triggered by the low glucose blood level. This would favour lipolysis, ketone body synthesis by the liver, the depot of triglycerides in the hepatocytes and finally a poor utilization of acetate (Schwalm & Schultz, 1976).
- The release of ketone bodies by the udder by the way of acetoacetate is a possible cause of ketosis if during high mammary activity, there is an increase in the blood levels of free fatty acids, acetate and betahydroxybutyrate (Kronfeld, 1972).

(iii) Abomasal displacement

Three theories attempt to explain these anomalies of the abomasum, yet none is entirely satisfactory (Coppock, 1974). The genetic theory attributes an increased mobility of the abomasum to selection of animals with a large gastric capacity. Another theory that of mechanical effect suggests the size of the pregnant uterus as the cause of displacement. With pregnancy, the abomasum is pushed forward and to the left beneath the rumen ; after uterine involution following parturition, the abomasum can no longer return to its previous position. In respect to a functional theory, all anomalies in abomasal position result from a prior atony of this organ. Numerous causes have been implicated : reflexes (stress during parturition, pyloroduodenal ulcers), toxins (endotoxins or histamine from suppurating areas, metritis, mastitis, pyelone-phritis), metabolic disorders (hyperketonemia, hypocalcemia, alcalosis). Interesting observations have been made on the role of high-energy fodders given during the pre- and post-partum period, indicating a relationship between overfeeding during the drying off period and abomasal displacement (Coppock, 1974). According to Svendsen (1969-1970), a high level of cereal concentrates in the diet increases the volatile fatty acids entering the abomasum and also local gas production. These volatile fatty acids reduce the motility of the abomasum which distended by the gas is then more easily displaced in the abdominal cavity.

(iv) Genital disorders

A wide range of clinical and epidemiological factors are involved in the etiology of genital disorders. During the development of steatosis, ketosis or abomasal displacement, placenta retention, metritis or mastitis commonly occur (Noordsy et al., 1974). In two dairy herds showing a high incidence of ketonemia, Peichev (1971) has observed an abnormally high incidence of dystocia, placenta retention and metritis and, in most of the animals, reduced fertility and a smaller number of calves born per year. Franzos (1970) succeeded in reducing the number of metritis cases in two stables where the disease was common, by reducing the energy and

nitrogen components of the diet to the strict minimum required. Sommer (1975) considers that post-partum metritis and mastitis is a direct consequence of metabolic disorders caused by incorrect feeding and then a perfect liver function is required to protect the animal against disorders. He thus tested hepatocyte activity during the eight weeks pre-partum by measuring the blood levels of total cholesterol and glutamic-oxaloacetic transaminase activity. In 1,000 cows considered as 'normal', this author found only 7 % affected by metritis, whereas of the 283 considered as 'abnormal' 72 i.e. 25 % contracted the disease.

Metabolic diseases and mineral nutrition

(i) Milk fever. Milk fevers occurs in the brief period between 72 or 96 hours pre- or post-partum and most frequently in cows at their fifth or sixth calving. The syndrome is characterized by muscular weakness and may be accompanied by lack of consciousness (coma). Low serum levels of inorganic phosphorus and varying levels of glucose, Mg and K may also occur. Other types of paresis usually termed 'downer condition' (Jönnson & Pehrson, 1969) may be distinguished from this syndrome. The exact mechanism of the hypocalcemia has not yet been specified but it is known that the phenomenon is initiated by the onset of lactation. The calcium requirements range from approximately 5 g per day, which is sufficient for the foetus, to between 13-18 g per day which is largely in excess of the 6 to 10 g available in the 'milieu intérieur' (Jorgensen, 1974).

A combination of the following factors : advanced age, diet and hormonal imbalance may explain hypocalcemia and hypophosphatemia. Older cows lose their apetite during the 4 days preceding parturition more easily than younger animals. In addition, they absorb less dietary Ca ; this is an important factor in the animal's predisposition to hypocalcemia because the Ca homeostasis depends largely at this time on increased absorption from the intestine (Kronfeld, 1971).

Since the findings of Boda & Cole (1956) that a high Ca intake pre-partum coincides with an abnormal incidence of milk fever, it has been shown that a limited amount of Ca (30 to 40 g per day) given before parturition followed by larger quantities after calving (140 to 190 g per day) provides an effective protection (Westerhuis, 1974). This is a way of maintaining parathyroid activity, thus facilitating bone resorption and gut absorption (Kronfeld, 1971). A high energy diet provokes not only the above-mentioned disorders, but also hypocalcemia at parturition because of fixation of the Ca in the adipose tissue (Luthman & Jönsson, 1972). Moreover the renal and hepatic steatosis which result from lipolysis could cause disturbances in vitamin D_3 metabolism (Lamothe et al., 1971) i.e. lowering of the level of 1-25-dihydroxycholecalciferol (Jorgensen, 1974) which thus reduces Ca absorption from the gut and its resorption from bone.

The hormonal changes linked to parturition or concomitant stress may also cause hypocalcemia. Hormones involved include oestrogens, glucocorticoids, prostaglandins, adrenocorticotrophic hormones and adrenalin. Contrary to previous thinking, changes in parathyroid secretion does not seem to play an important role in inducing hypocalcemia. In fact, it is difficult to explain the syndrome of hypoparathyroidism characterized by hypocalcemia and hypophosphatemia. The action of calcitonin on bone resorption and urinary phosphate excretion is better understood since the experimental induction of milk fever by intravenous administration of this hormone in lactating cows (Barlet, 1968) ; however the circumstances under which its secretion is disturbed by parturition remain unknown.

(ii) Tetanies. Hypomagnesemic tetanies are less closely related to parturition than the above-mentioned syndromes, but lactation does facilitate their incidence since the mammary output of Mg is in the order of 0.1 g per kg of milk (Todd, 1967). Grass tetany affects high-yielding dairy cows in the field during spring and autumn in relatively cold weather and when on grass still rich in nitrogen and potash (Samson, 1973 ; Wilcox & Hoff, 1974). Such conditions, combined with poorly regulated magnesium homeostasis, facilitate the development of hypomagnesemia, which is itself often accompanied by hypocalcemia.

The situation may be considered at three levels. In spring pastures the absorption of both Ca and Mg is reduced as nitrogen is freely available in the form of ammonium ions. There is an abundance of these ions as the nitrifying bacteria in the soil have been inactivated by the cold. Consequently less than 20 % of the Mg requirements of a lactating cow is provided.

Gastric reserves however are affected in that such grass is rich in ammoniacal nitrogen but depleted in carbohydrates following an activation of ammonia detoxification processes which require large amount of energy. Such food promotes a condition of rumen alkalosis which disturbs the Mg and Ca absorption with ensueing formation of ammonium-magnesium phosphates and phosphates of lime.

In the blood the levels of both Mg and Ca are lowered. In addition, the after-effects

of NPN metabolism (hyperammonimia, glutamine) may sensitize the nervous system to ionic imbalances. The resulting lack of energy results in a humoral syndrome similar to that seen in ketosis.

Regulation of metabolic disorders

During the drying-off period (6 and 8 weeks), cows in good condition should only receive the minimum of energy required, for both the animal and the foetus (7-8 UF), as reserves have been built up during the much higher yield of the middle three-months of lactation (Coppock et al., 1974). 'Steaming up', which is necessary to preserve the apetite of a dairy cow, should not be prolonged beyond 10-15 days prior to the expected date of parturition, nor should the animal be supplied with a higher energy ration than 1 % of body weight in concentrates at regular doses. With regard to the lability of gastric function at the end of pregnancy (Baird et al., 1974 ; Sommer, 1975), the basic feed of lactation should be continued during steaming to avoid disturbances in both appetite and digestion. Although the P-Ca ratio does not appear to play a decisive role in the prevention of milk fever, the daily ration of Ca during steaming should be controlled and adjusted to less than 50 g (Jorgensen, 1974 ; Westerhuis, 1974).

Throughout lactation the diet should be designed to satisfy the animal's requirements in relation to its production by improving its appetite and gut physiology. The following recommendations are important for the former point : distribution of a high-quality diet several times a day, e.g. 5 meals at 4-hourly intervals for a complete daily ration (Mayer, 1976), this because the feeding-time in the cow-bail is not sufficient for a high-yield animal to ingest the total amount of concentrate provided and required ; allocation of the daily ration according to 'production groups' (milk production 7,000 kg) or according to 'calving groups' (milk production 7,000 kg).

To improve digestion, it is important to : avoid any sudden change in nutrition during the first three months of lactation. When animals are put on rich pastures in the spring, Mg supplementation is recommended (Baird et al., 1974) ; it is good practice to supply a concentrate well balanced in vitamins, minerals, trace-elements and proteins (16-18 %) and a cereal level between 40 and 60 %. The remaining diet should be in the form of long fibres (Baird et al., 1974 ; Mayer, 1976).

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Summary of the discussion

The term "rumen rehabilitation" was explained: it embraces all therapeutic and prophylactic efforts applied in trying to retain the normal rumen metabolism, preventing any material (geometrical) or biochemical aberration. The speaker further explained that the graph on weight change has primarily a didactive function, and that further refining would disclose weight differences between lactations as well. In relation to the reported high NEFA levels following transport, Giesecke referred to similar observations on increased acetonaemia, leading even to decreased meat quality. High concentrate feeding before, and depression of neoglucogenesis during, transport would contribute to these effects.

THE PRACTICAL USE OF THE METABOLIC PROFILE TEST

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Summary

The Metabolic Profile Test is a diagnostic aid for production disease. It is based on a computerised interpretation of blood chemistry, but needs careful differential diagnosis for successful application. Seasonal changes in blood chemistry revealed by the test indicate periods of especial danger from production disease. Individual herd tests can be used either to elucidate the aetiology of outbreaks of metabolic disorder or to reveal unsuspected abnormalities in 'normal' herds.

Introduction

The Metabolic Profile Test is simply a diagnostic aid to show whether or not the blood chemistry of a dairy herd is 'normal'. The background work behind the test was carefully designed to ensure reliability and usefulness. The studies carried out included:-

1. An assessment of analytical error. (Manston & Rowlands, 1973).

2. Calculation of 'normal' reference standards were made both for mean values and their limits of variability. These are based on three surveys including blood chemistry from a total of 278 herds (Payne et. al., 1973; Payne et. al., 1974).

3. The precise method for carrying out the test was based on a simple statistical concept. Blood chemistry was shown to vary depending on a hierarchy of factors, first in importance being differences between herds. Thus, the test is basically a herd test involving an assessment of blood chemistry of milk yield groups within herds. Sufficient numbers of animals have to be sampled from each group - which in practice includes 7 dry cows, 7 mid-yielding cows in late lactation and 7 high yielding cows in peak lactation (see Rowlands & Pocock, 1976, for full background). It is worth pointing out that this system avoids the asymmetry of distribution of blood concentrations which is liable to occur in whole population results.

4. The results have to be displayed in a comprehensible way for ease of interpretation. A computerised system was developed, the programme for which is available (Payne, 1972; Rowlands & Pocock, 1971).

5. The results have suggested certain laboratory investigations many of which have been published - see for instance the effect of simple low protein status (Manston et al., 1975).

It cannot be too strongly stressed that the Metabolic Profile Test is a veterinary diagnostic aid in relation to production disease and not a test of nutritional adequacy. Many cows compensate for dietary errors by adjustment of milk yield etc. A sequence of algorithms has been compiled to assist in differential diagnosis. For instance, a diagnosis of protein deficiency is only fully valid if it is backed by evidence of low urea, albumin and haemoglobin concentrations. A high globulin resulting from an infection in the herd may depress albumin and lead to a spurious diagnosis unless all factors are taken into account. This need for differential diagnosis is one reason why so many components are included in the test and why three groups of cows are needed from each herd. The temptation to cut down on costs by using so-called mini profiles should be resisted because inevitably this leads to the risk of error in diagnosis.

Results and discussion

The practical use of the test.

1. General implications

Veterinary surgeons may request a profile test for two reasons. First; they may seek elucidation of an unexplained metabolic disorder within a herd. Secondly; although they may not know of any overt abnormality they may wish to check for hidden dangers which could lead to a future problem. As might be expected the incidence of 'abnormality' in the profile test is much higher in the first group than in the second (see table 1). Furthermore, when clinical problems do arise they can frequently be related to relevant abnormalities in the profile test.

Table 1. Relationship between 'abnormal' profiles and levels with clinical disorders.

Clinical disorder	'Normal' profile	'Abnormal' profile
None	13	17
Milk fever	0	10
Infertility	2	18
Ketosis	0	7
Poor milk yield (either quantity or quality)	1	7
	16	59

(For further details see Payne et al.,1973)

Useful implications can be gained from seasonal changes in the profile test results. Briefly these may be summarised as follows:-

(a) Blood glucose values tend to be low in late autumn and early winter which may reflect low energy status on late autumn pasture.

(b) Blood urea, albumin and haemoglobin are higher in summer than winter, which may reflect differences in protein status and nitrogen intake.

(c) Inorganic phosphorus (in lactating cows) and sodium values tend to be low in summer, which may reflect the low concentrations of these minerals commonly present in pasture herbage.

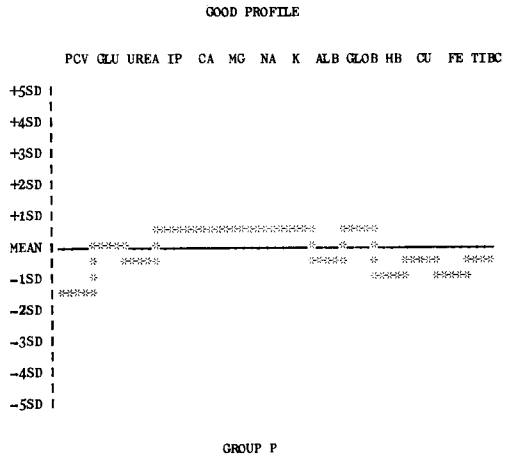
(d) Copper tends to be low in dry cows which may reflect the low copper status of maintenance diets and the importance of supplementation in the concentrates.

(e) Magnesium can be low in winter as well as in summer, indicating the need for supplementation in winter rations.

2. Interpretation of individual profiles

Typical profile patterns are displayed in Figures 1 - 8. The legends to these figures give details to help interpretation and differential diagnosis.

Figure 1.

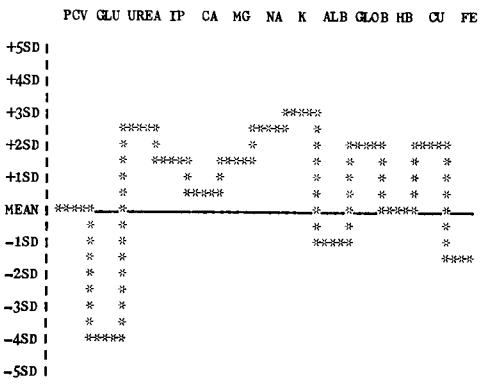


GROUP P

This was a small herd of about 60 cows kept by a really good stockman. He had a natural instinct for looking after his animals and although not feeding according to rules would offer extra food to his cows as and when he thought necessary. He was in fact much more scientific than he realised. He had a good relationship with his veterinary surgeon from whom he frequently sought advice.

Note that the profile pattern for this peak yielding group of 7 cows has all components well within the ± 2SD band. The profiles for the other two groups of 7 cows were also normal.

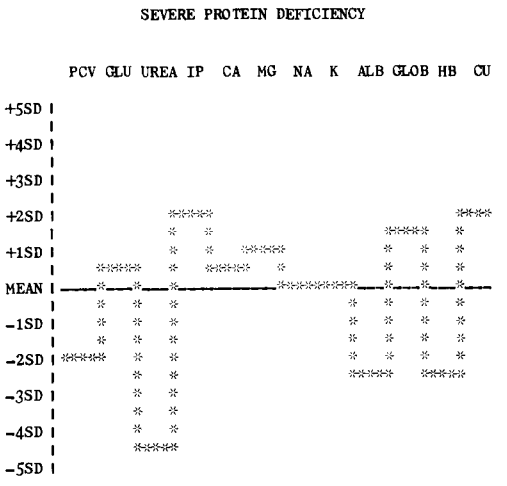
Figure 2.



GROUP 3

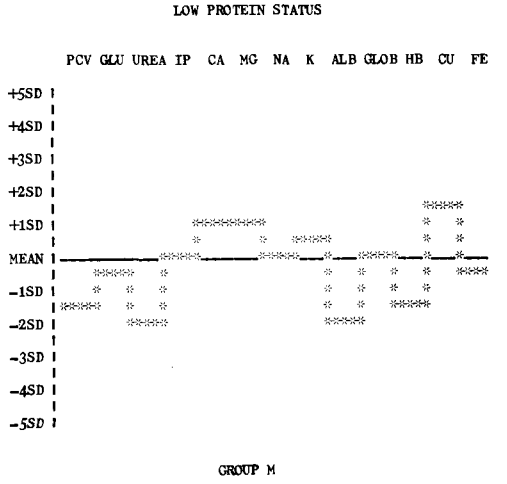
This was a large Friesian herd of about 140 cows. The herd had suffered a mild outbreak of ketosis the year before when a profile showed moderate hypoglycaemia. The severe hypoglycaemia now present was associated with overfat conditions at calving and liver failure.

Figure 3.



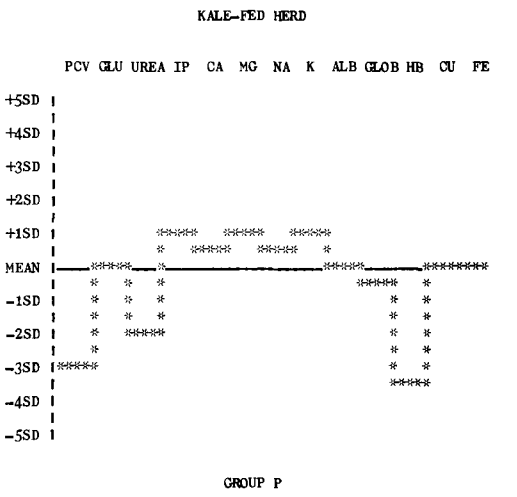
Urea concentrations are well below the normal standards of ± 2SD. PCV, albumin and haemoglobin are also low. This was an experimental group of cows at peak lactation. They had been fed a minimal protein intake of hay and barley containing approximately 10% crude protein. Milk yields were well maintained and there was little evidence of disorder. (For further details see Manston et al., 1975).

Figure 4.



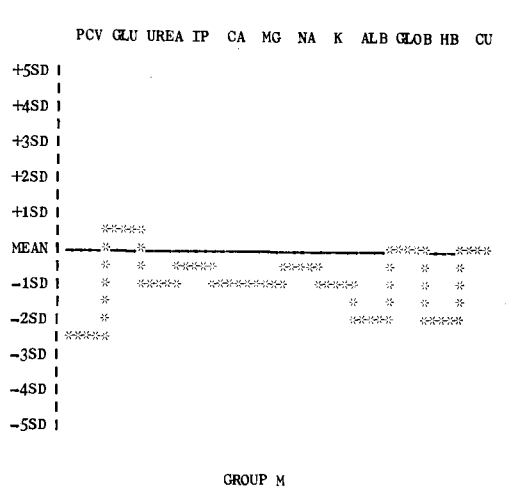
Profile of the same group of cows as Figure 3, but later in lactation. The urea, albumin and haemoglobin concentrations are returning to normal even though protein intake was still minimal. Later, when the cows were dry, the urea concentration remained low but all other components returned to normal.
(For further details see Manston et al., 1975).

Figure 5.



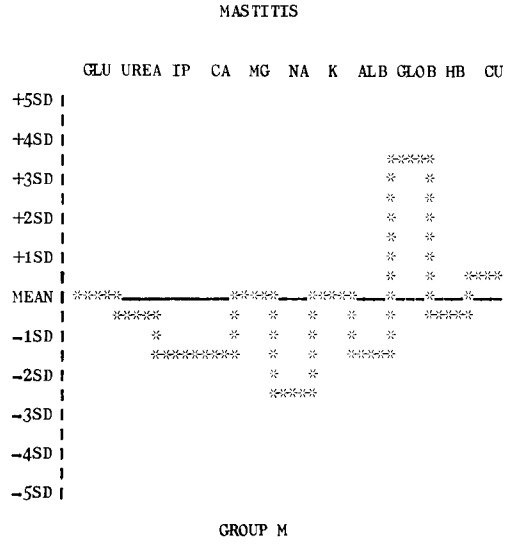
Profile from a herd suffering from kale anaemia. Note the low PCV and haemoglobin levels. Urea is low also, probably because haemoglobinuria had induced diuresis. The importance of this profile is that it should be distinguished from protein deficiency because albumin is not affected.

Figure 6.



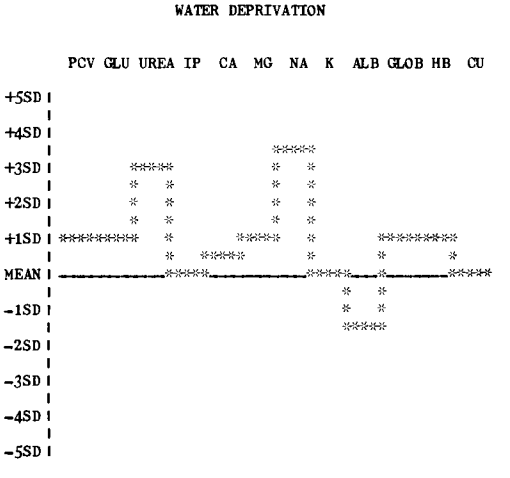
This is another profile, important again from the point of view of differential diagnosis. The herd was composed of about 120 Friesian cows grazing low lying land. It had had a history of moderately poor performance, associated with profile patterns indicative of low protein status. Protein intake on this pasture should have been more than adequate. Fluke infestation was confirmed by egg counts and liver enzyme tests. In some cases of this kind high urea concentrations are seen.

Figure 7.



Profile from a herd with an outbreak of mastitis. The bulk milk sample had a cell count of 1.5 millions, most cows were affected. Note the high globulin which tends to suppress albumin. Note also the low sodium, probably due to loss of sodium from the mastitic udder. These are important factors in differential diagnosis.

Figure 8.



This profile comes from an experimental group of lactating dairy cows in which water intake was restricted to 50% of normal for 4 days. Note the evidence of an early stage of dehydration with hyperuraemia and hypernatraemia. A more severe dehydration was shown to lead to progressive haemoconcentration with high PCV and haemoglobin concentration. From the differential diagnosis viewpoint the profile should not be confused with wasteful use of proteins which also shows hyperuraemia.

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Summary of the discussion

This paper caused many questions and remarks. Blood urea levels may serve to indicate the adequacy of protein and non-protein nitrogen utilization. Great care is needed however, in differential diagnosis since urea values can be interpreted in quite opposite ways. The example of "a bad profile" was produced by excessive "steaming up" before calving. The cause of the hypoglycemia was fatty liver. In the case of 10 herds with a high milk fever incidence the abnormalities were mainly in inorganic phosphate, magnesium and glucose. Abnormal calcium values are only rarely related because the test is not designed to detect the severe acute hypocalcaemia during clinical parturient disease. An apparently effective homeostasis prevents its use before this stage. A low concentration is defined as being less than 8.5 mg%, averaged in a group of 7 cows. The profiles of 50% of all normal herds show

some biochemical abnormalities, and this is about the same in the clinically affected herds. In the category of normal herds we find many which, on close examination, by professionals, must be classed as herds with subclinical production disease. In such cases, for instance if milk yield is suboptimal and hypoglycemia and/or hypomagnesaemia are confirmed, correction of the abnormality can give large improvements. For the present, indications as to abnormalities in the lipid metabolism cannot be included in the profiles, because of the considerable diurnal variation; so glucose appears to be the most reliable for diagnosis, although it is subjected to stasis. The proposal to include enzymes is sound, especially in so far as these assess the liver function. Geographical relationships with blood profiles do exist, for instance in magnesium and copper. The situation nevertheless is complex due to the interactions - for example magnesium is interrelated with phosphorus and copper is regulated by protein intake. Blood osmolarity has only been used experimentally. It is a most sensitive indicator of dehydration. Analysis of hair is not introduced because of some fundamental problems. The cost of profiles vary, depending on the follow-up consultations. Pasture herbage composition has a profound effect on the outcome of the profile, and reversely,wise handling of the profile can lead to conclusions on the right herbage composition.

PRACTICAL ASPECTS OF IMPLEMENTING A COMPREHENSIVE METABOLIC PROFILE ADVISORY SERVICE FOR DAIRY COWS

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Summary

Following the introduction of the "Compton Metabolic Profile" by the Agricultural Research Council, certain commercial organisations have set up a Metabolic Profile service using the computer programme, and field technique, advocated by the Compton group of workers, Institute for Research in Animal Disease.

BOCM SILCOCK set up such a service in 1973 and have conducted sixty six blood profiles.

In addition to the Compton Metabolic Profile service a Food Balance Sheet has been computerised which enables input data of certain essential nutrients to be estimated on the basis of full laboratory analysis of all feeds on offer to the dairy cow, together with on-farm records concerning feed intake.

An analysis of the results of the field experience in running a commercial Metabolic Profile service and supporting Food Balance Sheet is presented, and conclusions are drawn from the results concerning their efficacy as a field advisory technique.

A major characteristic of the BOCM SILCOCK service is the compulsory "interpretation session" which must take place on the farm when the results of the Metabolic Profile and Food Balance Sheet are available. This interpretation session is attended by the farmer, his veterinary surgeon and the nutritional adviser (cattle specialist) employed by the animal feed company.

The point is made that it is impossible to differentiate between advances in dairy cow nutrition solely as the result of the Metabolic Profile and Food Balance Sheet, as distinct from advances made as a result of better and more disciplined management, resulting from close collaboration of the farmer with his veterinary surgeon and nutritional adviser acting together as a team.

The paper concludes that techniques such as Metabolic Profiles can never be evaluated in respect of their cost/effectiveness in the laboratory. Instead wide scale analysis of field experience along the lines of that presented in this paper, are an essential pre-requisite of any overall practical and economic evaluation.

Introduction

The objective of this paper is to discuss the practical application of metabolic profiles and their value as an advisory aid in the field. For this purpose, an attempt has been made to quantify the "success rate" of profiles on a case study basis. It is hoped that the paper will serve to stimulate discussion by being as objective as possible It is not presented as an original scientifi paper and should not be interpreted as such. However, the object of agricultural research is to improve farming productivity. All attempts need to be made, therefore, to examine whether metabolic profiling of dairy herds fulfils this objective.

The productivity of the dairy herd is primarily dependent on the metabolic and nutritional status of each dairy cow. All to frequently, there is a tendency for high out put demands - milk and calves - to be associated with inadequate feed inputs, resulting in imbalances of body metabolites, which, if sustained, eventually become clinically apparent as "production diseases" such as hypocalcaemia and hypomagnesaemia.

The Compton Metabolic Profile technique, (CMP), as a means of monitoring the apparent status of representative animals from the herd in relation to feeding and management practice, is currently the focus of attentio as a promising management guide and has been adequately described elsewhere. (Payne et al 1972). Over the past six years, the Huntingd Research Centre alone (an independant U.K. Research Establishment) has handled some 250 profiles (Medd 1976) and the total number conducted nationally is probably in excess of 100. At the end of 1973, BOCM SILCOCK (a U.K. animal feed Company within the Unilever group) introduced the computerised "Food Balance Sheet" (FBS) developed as an adjunct to the standard CMP Test. The FBS was designed to complement the blood profile and to provide a more reliable guide to herd management. This paper, therefore, deals wit the conjoint use in the field of CMP's + BOCM Silcock FBS's.

The BOCM SILCOCK Food Balance Sheet (FBS)

In the past, it has been traditional practice for the nutritional adviser to collect samples of background feed for analysis of which the "nutrient balance" of the cow is assessed, and the winter feeding programme based.

The difficulties encountered when attempti to sample and quantify the intake of various uncontrolled feeds, (e.g. grazed grass, self fed silage), are obviously very great. This usually resulted in the situation in which only hay and silages were analysed, at low

sampling rates, while other important components of the diet (eg. minerals) were omitted. Such practices were obviously unsatisfactory, since the influence of feed intake on nutritional status cannot be overlooked. It is clearly advantageous to quantify all three variables in the sequential relationship:

NUTRIENT INPUT ⟶ METABOLIC THROUGHPUT ⟶ PRODUCTIVE OUTPUT

When proximate analysis of feed is combined with milk yield data, THROUGHPUT is omitted. When CMP's are interpreted solely in respect of the quantification of milk outputs (and stage of lactation) then data on INPUT are missing. All three are needed for optimal interpretation of the nutritional balance of the herd.

The FBS is conducted at the same time as the CMP blood test. Every feed available to the cows is listed - including such feeds as sugar beet pulp, barley and mineral licks - together with as accurate an assessment of intake quantity as possible. Representative samples are then collected. Although this is relatively straightforward in the case of rolled barley, sugar beet pulp etc, an auger is essential to obtain a core sample of silage, while a standard sample of grazed grass is achieved by thorough mixing of a large number of cut samples, and taking a representative sub-sample by quartering for submission for laboratory analysis. Thus blood samples from the 21 cows tested - 7 dry cows (Group 1), 7 low/medium yielders (Group 2) and 7 high yielders (Group 3) - and samples of each feed to which the cows have had access, arrive at the laboratory for analysis.

Data relating to the individual cows tested are also documented on the farm. These include an assessment of the animals' condition, liveweight, age, milk yield and state of lactation (see Table 1). An important point which is stipulated is that the herd should have been on the same feeding regime for at least three weeks prior to the test. This background information is essential for meaningful interpretation of the profile results.

From the documented data submitted, the theoretical requirements of energy (expressed as Starch Equivalent (SE)or Metabolisable Energy (ME)), Digestible Crude Protein (DCP) and minerals - Ca, P, Mg, Na, Mn and Cu - are calculated for each individual cow. Maintenance and production requirements for energy and protein are derived from the recommendations laid down in "Rations for Livestock", (Ministry of Agriculture, Fisheries and Food (MAFF) Bulletin 48) and are now being modified to encompass a change to ME (using MAFF Bulletin 33).

Table 1

ON-FARM DATA COLLECTION

FEED DETAILS

ALL feeds available to cows -listed and sampled.

Cow Details

Name/Number
Breed
Stage of Lactation (early, mid, late)
Condition (gaining, stable, losing)
Liveweight
Butter Fat%
Milk Yield
Feed Intake

Herd Details - total solids

Production requirement calculations involve three assumptions:

a. A minimum milk yield of 5 kg.
b. Production requirements are increased by 10% if the yield exceeds 16 kg for Jersey or Guernsey, or 22 kg for any other breed.
c. Animals in groups 2 and 3 have SE and DCP production requirements increased by 5% if in the first 100 days of lactation.

Maintenance and production requirements for Ca, P, Mg, Na (expressed in g/head/day) and for trace elements Cu, Mn (expressed as mg/kg of total diet dry matter) are based on the Agricultural Research Council (1965) recommendations.

The estimated nutrient intake is calculated by computer. Nutritional and dry matter intakes from the concentrates in the diet are calculated from feed analyses and the quantities fed (Table 2).

Table 2

CALCULATION OF NUTRITIONAL INTAKE FROM CONCENTRATES

Starch Equivalent

(SE)(kg) = kg fed x % ÷ 100

DCP (kg) = kg fed x % ÷ 100

Ca (g) = kg fed x % x 453.6 ÷ 100

P (g) = kg fed x % x 453.6 ÷ 100

Mg (g) = kg fed x % x 453.6 ÷ 100

Na (g) = kg fed x % x 453.6 ÷ 100

Mn (kg 1,000,000)= kg fed x mg/kg in feed

Cu (kg/1,000,000)= kg fed x mg/kg in feed

Dry Matter (kg) = kg fed x DM% ÷ 100

Bulky feeds of known intake are similarly processed. In order to estimate the dry matter intake from bulky foods such as self-fed silage in the winter months, and grazed grass during the summer, the computer first

calculates the dry matter intake capacity of the animal according to liveweight and milk yield. The dry matter intake resulting from consumption of concentrates and known quantities of bulky foods is then subtracted from this and the surplus regarded as the dry matter intake of the bulky food. Nutritional data are then calculated after this dry matter intake has been converted to intake "as fed".

Finally, the total intake of Energy (SE or ME), DCP, minerals and dry matter are added together for all feeds supplied to each individual animal. The average requirement and intake are calculated for each of these three groups and the differences shown as surpluses or deficits. These give a general indication of the apparent surplus/deficit of circulating blood metabolites (Table 3).

The FBS is interpreted in conjunction with, and not independently from, the CMP.

An example of the corresponding CMP to the FBS data tabulated in Table 3 is presented in Figure 1. The following comments therefore refer to both sets of information considered side by side.

In the example shown the most striking feature of the blood histograms is the very low blood glucose levels in all three groups. Since blood glucose can give a rough indication of the energy status of the animal, then one would expect to see the energy deficit. Table 3 bears this out, showing large deficits in energy for all the groups. Similarly, the small and non-significant deficits in blood Mg and Na indicated in the CMP are verified by the FBS. In addition, the FBS has identified a small protein deficit in Groups 2 and 3 which would not have been immediately obvious from the blood profile, although urea and albumin levels are a little below normal values.

Field Experience

Since the introduction of the CMP + FBS programme in the Autumn of 1973, 62 profiles have been conducted, for a variety of different reasons. These can be broadly categorised as:

a. Infertility problems
b. Metabolic disorders
c. Poor overall herd performance (non-specific)
d. Cows failing to obtain maximum yields at satisfactory levels
e. Post-partum problems
f. Routine test for either feed or health (Nothing clinically wrong)

In general, the majority of tests have been commissioned in an attempt to identify the causes underlying a specific problem(s) within the herd. Only 14 of the tests were conducted on a purely routine basis for herd health (category f).

The most common faults revealed by the tests were those related to mineral deficiency/imbalance. Of the 26 profiles which revealed mineral problems, 21 were conducted on herds in which symptoms were already apparent, mainly in the form of infertility. Corrective action was successful in 19 of these cases. Overall, recommendations arising from the results of the profile led to improved performance in 57% of the herds for which data are available, or in about 78% of the "problem" herds.(Table 4).

TABLE 3

BOCM SILCOCK — METABOLIC PROFILE SERVICE - FOOD BALANCE SHEET (FBS)

Farmer : Vet :

Total Milk Solids 14.68 — BOCM SILCOCK Cattle Adviser : John Brown

		SE kg/ cow / day	DCP	Ca	P g/ cow / day	Mg	Na	Mn mg/kg of diet Dry Matter	Cu
GROUP 1	7 cows								
	Mean Requirements	3.68	0.53	27.0	23.0	10.0	10.7	80.0	10.0
	Mean ration supplies	2.04	0.94	89.4	62.1	8.2	9.7	102.1	20.0
	Surplus	0.0	0.41	62.3	39.1	0.0	0.0	22.0	9.9
	Deficit	1.64	0.00	0.0	0.0	1.8	1.0	0.0	0.0
GROUP 2	7 cows								
	Mean Requirements	6.08	1.09	47.5	33.6	14.2	14.6	80.0	10.0
	Mean ration supplies	4.58	0.98	92.3	59.2	12.6	13.7	100.0	18.2
	Surplus	0.0	0.00	44.7	25.6	0.0	0.0	20.0	8.1
	Deficit	1.5	0.11	0.0	0.0	1.6	0.9	0.0	0.0
GROUP 3	7 cows								
	Mean Requirements	9.94	1.97	78.7	50.1	20.3	20.4	80.0	10.0
	Mean ration supplies	8.44	1.81	144.9	82.6	17.3	20.0	95.5	16.1
	Surplus	0.0	0.00	66.2	32.4	0.0	0.0	15.4	6.1
	Deficit	1.5	0.16	0.0	0.0	3.0	0.4	0.0	0.0

TABLE 4 BOCMS METABOLIC PROFILE & FOOD BALANCE SHEET SERVICE

Total number of profiles conducted			62
Number for which data are available -	No.conducted on routine basis	14	
	No.conducted to investigate problem(s)	40	54
No. in which no corrective action taken as result of profile		9	
No. in which performance was improved as result of action -			
	fertility & breeding	15	
	milk yield & quality	6	
	health	5	
	general performance	5	31
No. in which performance not improved		8	
No. in which performance cannot yet be fully assessed		6	54

Examination of the blood profiles of the 62 tests conducted by BOCM SILCOCK in respect of blood parameters glucose, haemoglobin, serum urea and albumin reveal seasonal trends in the concentration of these metabolites (Table 5) reflecting overall energy and protein status. These trends are in broad agreement with the observations of Payne (1972) although Payne had data on a much larger survey of dairy herds. In general, glucose concentrations increased during the Winter and declined substantially during the Summer months, especially June - August. Urea and albumin concentrations showed the reverse trend, being greater in the Summer than in the Winter, while haemoglobin concentrations were rather lower in the Winter compared to the Summer, reaching a peak value of 12.96 g/100 ml in September.

In the majority of the profiles conducted, low blood glucose levels were associated with low milk yield and quality, infertility problems and some incidence of ketosis in milking cows. In most cases, performance was improved by making adjustments to the feeding levels, and inclusion of extra limiting minerals in the feed programme. In other cases, in which high protein intake was implicated in conjunction with low blood glucose levels, it frequently transpired that the herd did not have access to adequate water supplies. This was often reflected in high PCV percentage, and high Na and K levels, since excesses of these components excreted via the kidneys when adequate water is available. High blood glucose levels were sometimes associated with digestive disorders and poor milk quality, and, in some instances, associated protein status was also found to be low.

In addition field experience has shown that high urea levels in conjunction with low/normal globulin levels, are frequently indicative of a situation in which the cows have had access to pasture to which fertiliser has recently been applied, resulting in excessive N intake. Low levels of haemoglobin and albumin, which decline in concentration with milk yield, are often indicative of the presence of liver fluke, although the same patterns can also indicate a deficiency of protein.

In general, the CMP and FBS data indicate that protein deficit had a much greater detrimental effect on milk production and health than an excess of protein in the diet.

Confounding effects of Profiles, Balances and General Advice of Management

Our experience in the field has shown that the inter-disciplinary approach to the interpretation session following the profile, in which veterinary surgeon, nutritionist and farmer combine their efforts to decide on the necessary course of action, is an essential

TABLE 5

Month	BLOOD PARAMETER (average values) Blood Glucose (mg/100 ml)	Serum Urea (mg/100 ml)	Serum Albumin (g/100 ml)	Blood Haemoglobin (g/100 ml)
January	45.50	13.78	3.36	11.74
February	45.67	14.84	3.19	11.41
March	46.30	15.11	3.49	11.13
April	45.68	12.18	3.30	10.80
June	40.81	28.16	4.36	11.90
July	35.15	16.86	3.52	11.83
August	32.11	21.19	3.51	11.52
September	43.94	23.54	3.45	12.96
October	47.78	17.02	3.22	11.57
November	45.93	15.46	3.00	11.89
December	44.73	13.52	3.36	12.12

pre-requisite if the deficit/surpluses identified in the blood analysis and Food Balance Sheet are to be rectified without further disruption to production. It is quite impossible to distinguish between the improvements resulting from the computerised outputs of nutritional information on the one hand, and the considered advice given to, and accepted by, the farmer on the other. The combination of the two together is all-important, since information without interpretation, and advisory discussions without factual information, are equally ineffectual.

These comments are pertinent to any academic discussion on the merits or demerits of blood profiling. It can be argued that many of the scientific bases for the CMP are hypothetical. For instance, the question is not answered as to what constitutes "abnormality' and what blood concentrates are considered "normal".

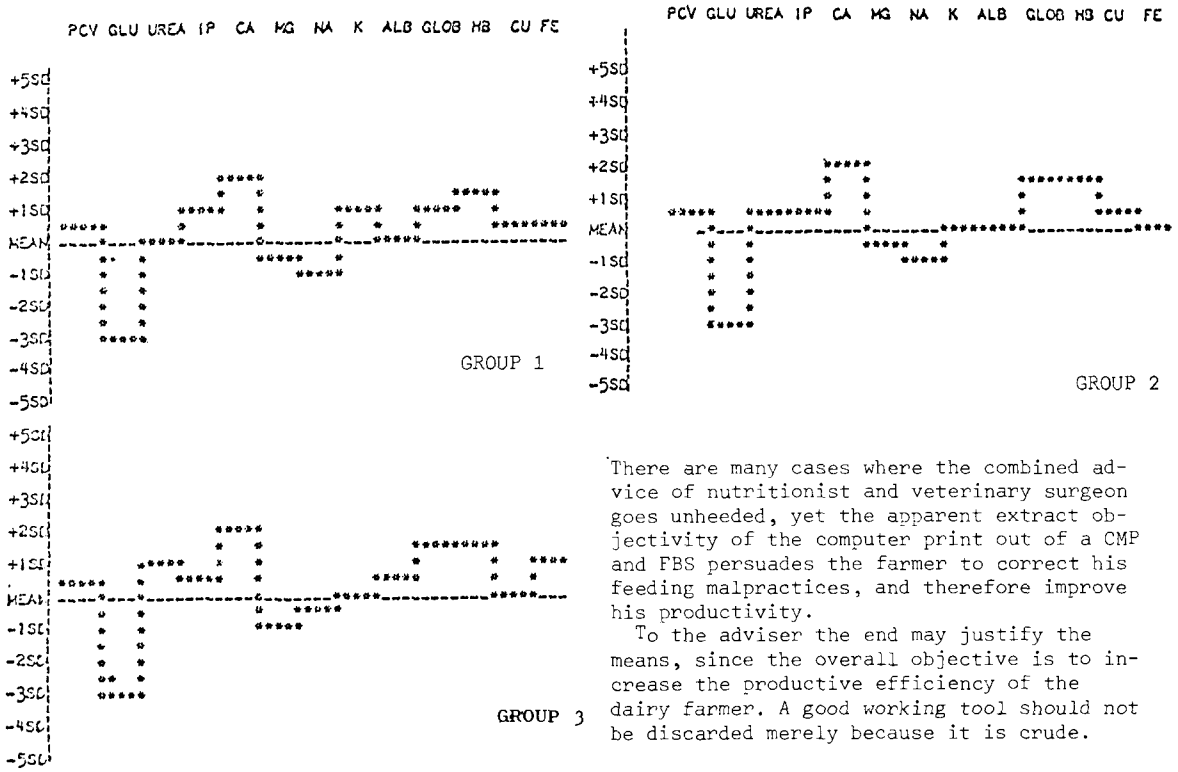
Again, it is easy to interpret low blood glucose levels as indicating energy deficit, but Annison (1976), in a study of glucose biokinetics, using the isotope dilution technique, has shown that blood glucose concentrations are not directly correlated to either glucose entry rates (from the alimentary canal) or to milk yield or lactose output.

Furthermore, the various indicators of protein status (haemoglobin, albumin, globulin, urea) are incapable of direct interpretation in terms of either total N entry rate or of "protein reserves" within the body mass.

On the other hand, field experience shows that most herds having low blood glucose levels also have energy deficits on the FBS, and correction of low energy inputs usually leads to a restoration of blood glucose levels to more normal values and to improved milk yield. In other words, in spite of the dubious scientific foundation of certain aspects of the CMP, the test seems to be a useful advisory aid in the field, although several parts of the programme are obviously capable of revision and improvement.

Finally, it can be argued that it does not require the conduct of a complete and costly CMP to detect such elementary feeding errors as energy or protein imbalance. A competent nutritionist could be expected to detect and correct such factors by less sophisticated and less costly devices. On the other hand, it is one thing to detect a feeding error, but quite another thing to persuade a dairy farmer to alter his feeding programmes, especially if added cost is entailed.

Figure 1



There are many cases where the combined advice of nutritionist and veterinary surgeon goes unheeded, yet the apparent extract objectivity of the computer print out of a CMP and FBS persuades the farmer to correct his feeding malpractices, and therefore improve his productivity.

To the adviser the end may justify the means, since the overall objective is to increase the productive efficiency of the dairy farmer. A good working tool should not be discarded merely because it is crude.

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Summary of the discussion

The answers of Prof. Wilson to critical queries related to the cost of metabolic profiles and the use of them for mineral studies are given in unabridged form.

1. The metabolic Profile and Food Balance Sheet is one of the management aids made available "at cost" (i.e. no profit taken) by my company. It is only provided to large dairy farms (more than 100 cows) with better-than-average milk yields (over 5000 kg). The farmer, the veterinary surgeon and the company nutritionist must all agree that the expense on the test is worthwhile and all three must agree to attend an "interpretation session" on the farm when the results of the test are available. The average cost of conducting the test varies, since the veterinary surgeon makes his own charge for bleeding the 21 test cows, but it averages 100 Pounds, or 1 Pound per cow in the herd. To recover this money an extra 13-14 kg of milk must be obtained. As I stated in my paper, 78% of the farmers with "problems" considered that they obtained a more than sufficient benefit from the test to justify their monetary outlay. There is no thought at present at making the test more widely available to average or below-average farmers where simpler techniques suffice.

2. An attempt is made to take account of availability of minerals from different sources, different grass growth seasons and different age of cow, but there is insufficient information available for us, or anyone, to carry out such an exercise with the accuracy one would desire. In the case of gross deficiencies or excesses, the answer is unlikely to be affected. In the case of marginal excesses or deficiencies, the answer might be interpreted along with the blood profile data which, by definition will deal with "available nutrients" and not only with "ingested nutrients". This is an example of the principle given in my paper that it is necessary to know both the "inputs" and the "throughputs" since the data relating to only one can at times be misleading.

If the "complete diet feeding system" were to be introduced, there should be no difficulties in determining the general and mineral requirement of groups of animals since all information would be on hand.

INPUT/OUTPUT RELATIONSHIPS OF DAIRY COWS WITH PARTICULAR REFERENCE TO THE LAW OF DIMINISHING RETURNS

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Summary

This paper considers the logistics of the relationship between concentrate feed intake into dairy cows and their resultant milk output. The data are drawn from several sources - the computerised records of over 1,300 herds participating in BOCM SILCOCK dairy herd management scheme ("Dairy Enterprise Plan"), from comparable data produced by the MMB of England and Wales (LCP Scheme), and from a single high yielding herd.

Interpretation of herd data is difficult because neither milk output nor feed input are in practice clearly differentiated between the dependent and independent variables in the statistical analysis. Various ways of tackling this problem are discussed, and data are presented showing the different apparent responses when herds are grouped according to yield and different rates of feeding examined compared to when the same herds are grouped according to feed input and the resultant milk yields compared.

The paper finally presents evidence of looking at the same herd, under relatively unchanged levels of management, over consecutive time periods in order to examine the marginal responses to marginal differences in feeding rate. This technique is applied to a well recorded high yielding herd and indicates that the law of diminishing returns does not result in diminishing responses to nutrient input at yield levels around 7205 kg/cow/year.

Introduction

The most important and costly input into the dairy herd is animal feed, and the most economically important of the two outputs (calves and milk) is milk yield.

The ratio of feed input to milk output is often conveniently measured in economic terms as "margin over concentrates". This is defined as the difference between the value of milk sold per cow per year and the cost of bought-in feeds per cow per year. This ratio is correlated to the gross margin per cow per year, which in turn is correlated to profit.

However, the above ratio is not easily expressed in nutritional terms on a herd basis. The total nutrient input is rarely known with any precision, and complicated devices are needed to quantify this parameter, especially where cattle are "self fed" as in the grazing situation (Wilson, 1976).

Although an attempt is made to ration most well-managed herds each winter, most schemes are based on assumed feed availabilities for an assumed winter period at an estimated mean milk yield. Deviations from the assumptions employed are rarely recorded with any accuracy.

Feed input data are, however, accurately known in respect of bought-in feeds. The nutrient content of such feeds can be obtained without difficulty and most compound feeds are made to constant specifications in which the nutrients theoretically required to produce a given quantity of milk are contained in a stated quantity of compound.

However, there are several important factors which confound any simple overall linear relationship between feed (or nutrient) intake and milk output. Firstly, the relationship is modified by stage of lactation. Under- or over-feeding in early lactation has a long-term carry-over effect, at least until the end of that lactation if not beyond (Broster, 1974). Secondly the utilisation of nutrients is partitioned between milk output and liveweight change. Although the between-cow differences in apparent digestibility of nutrient is relatively small, the differential partitioning of nutrients is of major importance, and adds considerably to the between-cow and between-herd variation in milk yield. Thirdly, the conversion of nutrients to milk is subject to the law of diminishing returns. As cows approach their genetic ceilings for yield the provision of extra nutrients will be converted into extra milk at progressively lowered efficiency. Fourthly and lastly, the effect of supplying extra compound feed is confounded with the varying quantity and quality of "background" forage. This point is obviously very relevant to herds producing most of the milk during the summer when cows are grazing grass of different quantity and quality as the season progresses. It is also relevant to winter milk producing herds, since it is rare for such herds to be fed on identical quality hay or silage throughout the winter period.

Although, therefore, it is difficult to calculate meaningful ratios of feed input to milk output on a practical herd basis, it is nevertheless important for the farmer to know whether or not the provision of extra feed will produce a more than commensurate return in additional milk output. Such considerations have an immediate bearing on overall herd

feeding policies and also on the setting of milk yield targets. This paper presents data relevant to this topic on a herd basis, derived from herds in which accurate records of total milk yield and total bought-in concentrate feeds (e.g. barley, sugar beet pulp, proprietary compound feeds) are kept.

Data from 1,339 BOCM SILCOCK Recorded Herds

Data are presented for 1339 herds (excluding Channel Island breeds) for recording years ending in 1975. (The "recording years" are staggered for obvious logistical reasons, so the data refer to 12 month periods ending at any time within the calendar year 1975). Table 1 presents the data sorted by yield group. (The actual yield intervals employed are steps of 100 Imperial gallons but these have been transformed in the table into metric equivalents, using the conversion factor 1 gallon milk $\simeq$ 4.68 kg milk).

Table 2 presents the identical data resorted by total bought-in concentrate feed usage.

It will be noted that there is an obvious linear regression of both milk yield on concentrate intake (Table 2) and also on concentrate intake on milk yield (Table 1). However, the regression coefficients differ according to which factor is regarded as the dependent and which is regarded as the independent variable. To illustrate this important point, by comparing the overall difference in milk yield across the 8 classes in Table 1 with the overall differences in concentrate intake, it will be seen that the 3478 extra kg of milk are obtained at the expense of an extra 1440 kg of concentrates, or a ratio of 0.4:1 kg milk. However, by comparing the differences in the same two parameters in Table 2, the extra 1380 kg of concentrates has only produced an additional 1802 kg of milk, which produces a very different ratio of 0.77:1 kg milk.

The reason for this apparently conflicting result is that neither milk output nor feed input can be classified as dependent or independent variables. Milk might be expected to be dependent on feed input but in practice feeding rates are often determined by the previous milking performance of the cow.

Again, many other important managemental factors are confounded by the grouping of herds by either yield or feed input. Higher yield levels tend to be confounded with superior management. This is illustrated in Table 1 by lower dry cow percentage and, to a lesser extent, with a slightly higher percentage of winter milk.

On the other hand low concentrates usage tends to be confounded with greater emphasis of self-sufficiency and reliance on grass productivity. There is a tendency to let grass output dominate cow yield rather than an attempt to exploit the genetic potential of the cow for milk production by means of supplementary feeding.

In both Tables 1 and 2 the overall mean conversion of concentrates into milk is 0.32 kg/kg milk. In Table 1 this ratio remains fairly constant across most columns in the table until yield levels of 6085-6550 kg are reached. A very different picture emerges from Table 2, when feed is deemed to be the independent variable. Here there is a two-fold difference in mean feeding rates across the table, from 0.18 kg/kg milk to 0.39 kg/kg milk, although the overall mean value of 0.32 kg/kg milk obviously remains unaltered.

It will be seen, therefore, that it is not possible, from survey data of this type, to calculate marginal conversion rates of feed into milk at marginal levels. There is a tendency for the higher yielding herds to require disproportionately extra feed for the extra gallons, but on overall means the conversion only widens from 0.32 kg/kg milk to 0.37 kg/kg milk for herds giving over 6550 kg.

TABLE 1 Yield Groups (Excluding Channel Island Herds)

	kg Milk Yield								
	<3745	3745-4210	4210-4680	4680-5150	5150-5620	5620-6085	6085-6550	>6550	Whole Sample
	<800	800-900	900-1000	1000-1100	1100-1200	1200-1300	1300-1400	>1400	(gallons)
Number of Herds	57	145	280	379	282	142	44	10	1339
Average number of cows in herd	88.4	90.8	97.2	93.7	93.2	93.8	79.9	67.7	93.1
Dry cow %	22.8	19.6	18.6	17.6	16.7	16.1	15.4	14.5	17.8
Milk Yield/ cow (kg)	3416	4015	4460	4910	5354	5826	6280	6894	4905
Winter Milk %	46.9	43.2	48.4	47.9	48.4	49.0	51.8	47.6	47.9
Concentrates / cow (tonnes)	0.96	1.19	1.31	1.46	1.64	1.81	2.04	2.4	1.58
kg concentrates /kg milk (kg)	0.28	0.31	0.31	0.32	0.33	0.33	0.35	0.37	0.32

Source: BOCM Silcock Dairy Costings, 1975

TABLE 2 Concentrate Usage (Excluding Channel Island Herds)

	Concentrates Fed Per Cow (Tonnes)						
	<0.75	0.75-1	1-1.2	1.2-1.5	1.5-1.7	>1.7	Whole Sample
Number of herds	20	97	266	302	313	341	1339
Average number of cows/herd	84.5	89.2	102.4	92.2	92.4	89.0	93.1
Dry Cow %	22.2	19.7	18.4	18.2	17.2	16.7	17.8
Annual Milk Yield / Cow (kg)	3660	4184	4525	4797	5026	5462	4905
Winter Milk %	45.9	44.0	46.0	48.3	47.9	49.9	47.9
Concentrates / cow (tonnes)	0.62	0.89	1.12	1.36	1.58	2.00	1.48
kg concentrates/kg milk	0.18	0.23	0.27	0.28	0.34	0.39	0.32

Source : BOCM Silcock Dairy Costings, 1975

The long-term time trends for this parameter presented in Table 3 produce a similar result. Although this table confounds annual variation in the climatic and biological factors governing milk production, such as grass growth, nevertheless the data indicate a small marginal rise in feeding rate, from 0.29 kg/kg milk in 1963 to 0.32 kg/kg milk in 1975, with most of the intervening 11 years intermediate. During the same period mean milk yields rose from 4329 kg in 1963 to 4905 kg in 1975.

Throughout all this section the intake of concentrate feed has been considered in isolation. In practice, concentrate and forage usage are inter-dependant and as concentrate input increases it is usual for forage input to decrease, although less than correspondingly (Balch and Campling, 1970). As a consequence there is another unrecorded confounding factor involved in Tables 1 and 2, namely the "background" forage consumption.

TABLE 3 Recorded Herd Data 1963 - 75

	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975
Number of Herds	327	326	369	434	439	420	425	498	907	643	1010	1060	1339
Average Number of cows per herd	38	40	43	45	49	53	59	66	71	77	86	92	93
Milk Yield / cow (kg)	4329	4230	4306	4399	4366	4493	4516	4483	4647	4731	4788	4816	4905
Concentrates / cow (tonnes)	1.19	1.17	1.24	1.21	1.22	1.26	1.31	1.30	1.36	1.42	1.43	1.38	1.48
kg concentrates/kg milk	0.29	0.29	0.31	0.29	0.28	0.28	0.31	0.31	0.31	0.32	0.32	0.31	0.32
Forage hectares / cow	0.63	0.62	0.61	0.58	0.54	0.52	0.52	0.53	0.50	0.48	0.51	0.49	0.47

Source : BOCM Silcock Dairy Costings, 1975.

Since forage input is likely to have decreased as yield and concentrate usage increased, the slight increase in the feed: milk ratio must be interpreted with caution, because more concentrates would be required to make up for that portion of the maintenance and production requirement provided by forage in the lower yield groups.

Lest the data pertaining to the BOCM SILCOCK recorded herds presented in Tables 1-3 be considered biased, further supporting data are presented in Tables 4-5, relating to the Milk Marketing Board of England and Wales. The same general observations apply equally to these tables derived from this independent source.

Data from the same herd recorded in successive years

In order to remove some of the more obvious variables confounding the data in Tables 1-5, the changes in milk output and feed intake encountered in a single herd in successive years have been examined. Here such factors as management, milking efficiency and genetic capacity of the cows have been constant or nearly so throughout, but the confounded seasonality factor, relating to different years is still a problem. The main interest in these studies is the manner in which the concentrate feed:milk ratio changes at progressively higher yielding levels, so the high yielding herd of 60 cows at the Knaptoft Demonstration Farm, situated in Leicestershire, has been selected as an example.

Table 6 presents data for the five years 1971 to 1975 inclusive. It will be noted that yield progressively increased over this period, from 5570 kg in 1972 to 7296 kg in 1975. The marginal increases in both concentrate feed and milk for each of the 3 paired years are shown in Table 6, from which it is seen that the conversion ratios of marginal feed input to marginal milk output varied from a low 0.33 kg/kg milk in 1973/74 to a high of 0.56 kg/kg milk in 1974/75.

TABLE 4 Herd Analysis by Yield Groups (All herds non Channel Island)

kg milk per cow	<3275	3275-3745	3745-4210	4210-4680	4680-5150	5150-5620	>5620
(gallons milk per cow)	<700	700- 800	800- 900	900-1000	1000-1100	1100-1200	>1200
No. of Herds	95	191	366	505	394	205	57
Average number of cows/herd	51	55	63	69	71	70	69
Yield (kg/cow)	2916	3548	3992	4450	4881	5340	5845
Concentrates/cow (tonnes)	0.99	1.18	1.30	1.46	1.60	1.76	1.96

Source: Milk Marketing Board LCP Services, Dairy Herd Analysis, 1973/74

TABLE 5 Analysis by Concentrate Use (All herds non Channel Island)

	Concentrate Use (Tonnes)					
	< 0.75	0.75-1	1-1.25	1.25-1.5	1.5-1.72	> 1.72
Number of Herds	63	152	321	464	376	437
Average number of cows/herd	58	64	68	66	69	64
Yield (kg/cow)	3477	3795	4170	4362	4610	4867
Dry cow%	21	18	18	17	17	16
Concentrates / cow (tonnes)	0.59	0.88	1.11	1.35	1.60	2.00

Source: Milk Marketing Board LCP Services, Dairy Herd Analysis, 1973/74

The overall conversions (total feed into total milk) were 0.35, 0.36, 0.35, 0.37 respectively in the four years.

These data illustrate how, within a single small herd, the between-year variation in feed:milk ratio is of a much higher order of magnitude than in the case of the survey data considered earlier. The low marginal conversion of 0.33 kg/kg milk refers to a year of good grass growth and an excellent silage crop. Milk production derived from the "background" feeds was therefore correspondingly high, and the contribution required from the concentrate portion of the diet was lower than estimated when the winter feeding programme was fixed. Opposite arguments apply to the preceding and succeeding years.

Feeding additional concentrates is clearly economic as long as the value of the extra milk produced more than compensates for the extra cost of food consumed. Table 6 calculates both these parameters, and indicates that, in all 3 paired years, the additional milk production was highly profitable.

TABLE 6

KNAPTOFT RESULTS 1971-1975:COST OF EXTRA MARGINAL kg MILK

Factor	1972	Diff	1973	Diff	1974	Diff	1975
Yield (kg/cow)	5569	506	6075	575	6650	646	7276
Concentrates (tonnes)	1.93	0.27	2.20	0.19	2.39	0.36	2.68
Feed conversion (kg feed/kg milk)	0.35		0.36		0.35		0.37
Feed conversion of Extra kg of milk		0.53		0.33		0.56	
Cost of Concentrates (p/kg)		2.42		2.41		4.29	
Value of Milk (p/kg)		4.68		5.77		7.52	
Profit on Extra marginal kg milk (p)		2.26		3.36		3.23	

However, because of the operation of the Law of Diminishing Returns, this Knaptoft herd must eventually reach a point where the cost of extra feed input is no longer fully compensated by the value of additional milk output. We do not know when this point will be reached, and there are too few critical studies with high yielding herds to assist in predicting the limiting milk yield in this respect. Much depends on the genetic capacity of the cows, since in a well managed herd it should be genetic rather than managerial or environmental factors that determine the eventual break-even point. This point is illustrated schematically in Figure 1, which shows how 4 herds, differing in genetic potential for milk production, would be expected to have 4 separate Diminishing Return curves, each with different maxima and therefore, with different points (illustrated by short, thick arrows) when a 10% return on the extra marginal monetary outlay on additional feed would be achieved.

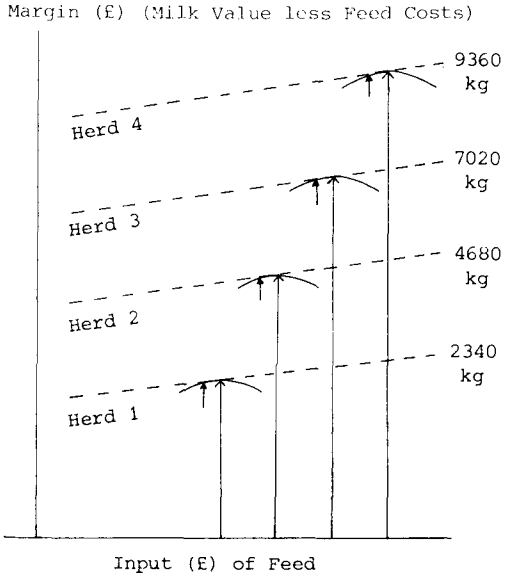

Figure 1

It is statistical considerations such as these that should enable the individual farmer to answer the very complex, but seemingly simple question, "Does it pay to feed additional concentrates to cows for extra milk production?"

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ANEMIA IN DAIRY CATTLE; ITS INCIDENCE AND RELATIONSHIP TO THE METABOLIC PROFILE

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Summary

The incidence of anemia in dairy cattle was determined in over 7,000 cattle throughout England that had metabolic profiles done over a period of several years. The incidence of anemia was categorized by age and relative level of milk production. The anemia was most severe in the winter months and in higher producing dairy cattle.

In an effort to determine the cause of the anemia in these dairy cattle, anemic herds were evaluated compared to normal control herds throughout the Spring of 1973. Approximately 50 herds were evaluated including 25 anemic herds and 25 controls herds. Several causes of anemia were determined including chronic infection as evidenced by hypergammaglobulinemia; a mild hemolytic anemia associated with the feeding of rape and kale; and the anemia associated with inadequate protein intake reflected by low serum albumins. Serum iron and total iron binding capacity was determined and at no time was there evidence of iron deficiency anemia in any cattle. The levels of iron did not reach the low point associated with iron deficiency anemia in calves. Anemia was not shown to be detrimental to milk production, to time of first heat, to milk solids-no-fat nor to milk fat.

Introduction

Anemia in dairy cattle is a common finding that has been poorly characterized (Whitlock et al., 1974). It was the purpose of this investigation to ascertain the seasonal, milk yield and age incidence of anemia in British dairy cows (5).

Since much of the anemia in woman, pigs, and calves is related to iron deficiency, a study was undertaken to determine the relationship of anemia in the adult cow to an iron deficiency. Nine cows of mixed breeds were used for this experiment. They were monitored daily for three weeks, then blood was removed three times per week for six weeks. The total amount of blood removed was equivalent to their blood volume. Five of the nine cows were given sufficient elemental iron as iron dextran to replace that amount of iron removed during phlebotomy. The cows were monitored an additional ten weeks to follow their recovery. Weekly metabolic profiles (Payne et al., 1973) were done throughout the entire experiment. Additionally, hematological parameters (PCV, MCV, Hb, MCHC and RBC) were determined three times weekly (immediately prior to bleeding each animal). Serum iron and total iron binding capacity was determined weekly throughout the experiment.

Since liver flukes are commonly found in British cattle and flukes can cause anemia, five known fluke infected herds (as determined by necropsy lesions of herdmates and fecal egg counts) were monitored by metabolic profile and hematological parameters. The purpose was to characterize the anemia in the fluke animals and compare it to other types of anemia.

Additionally, twenty-five herds of cattle throughout southern England were selected as having anemia from metabolic profiles that had been done earlier. These herds were visited a second time to evaluate milk records, breeding charts and to obtain blood for a second metabolic profile and to further define the hematologic status. Herds which had been involved in the study to evaluate the seasonal changes in the metabolic profile served as controls. Approximated two herds were evaluated each week during January, February and March of that year.

Results and Discussion

The effects of season and milk yield on the hemoglobin concentration are shown in Table 1.

The reason for this clearly defined seasonal incidence is not immediately apparent but may be related to Brassica spp. feeding which is a common practice in the late autumn and early winter. Certain of these species may cause hemolytic anemia (Greenhalgh, 1969). Cows giving the most milk had the lowest hemoglobin concentration. Age had a significant effect on the incidence of anemia. High yielding cows over 9 years old were more prone to anemia than others which was most significant ($P < 0.005$) during the winter season. The 2-3 year old low yielding cows were also more prone ($P < 0.01$) at this time than others giving similar amounts of milk. These findings are in agreement with one report (Kitchenham) but differs from another (Wingfield and Tumbleson). This relationship in the later report may not have been detected due to the few cattle studied. The inverse relationship of hemoglobin to milk yields had been noted previously (Payne et al, 1973). However, the reason for the relationship is unclear and it is not known if low

hemoglobin concentrations are detrimented to milk yield.

The phlebotomy experiment showed no statistical differences between controls and cows given iron dextran as regards the blood glucose, and serum concentrations of urea, sodium, potassium, calcium, phosphorus, magnesium, total protein, albumin, and copper. Similarly no differences between groups were found in hemoglobin concentration, mean cell volume or mean corpuscular hemoglobin concentration. As expected the hemoglobin decreased in all cattle 3-5 gms/dl while the mean cell volume increased 5-15 cubic microns. The mean corpuscular hemoglobin decreased from an overall mean of 34% to 31%. Most interesting was an increase in serum iron in both groups of cows from an initial mean of 160 to 250 mg/dl. The total iron binding capacity increased from 411 to 480 mg/dl. As a result the % saturation of iron increased from the initial mean of 41% to 59% during the maximum hematological response.

Table 1.Milk yield and seasonal influence on the incidence of anemia.

season	milk yield 0-7 kg	8-16 kg	16+ kg
April-July	0.4% (564)	3.0% (624)	5.1% (747)
August-November	2.4% (628)	6.6% (608)	9.8% (680)
December-March	8.3%* (927)	16.8% (888)	24.6%** (1408)
total cows	2119	2120	2836

*p < 0.01 greater incidence of anemia in 2-3 yr olds

**p < 0.005 greater incidence of anemia in cows over 9 yrs old

% indicates that proportion of cows with a Hb concentration equalor less than 9.8 gms/dl. The number in parenthesis is the number of cows in that category.

Surprisingly, the phlebotomy experiment gave no differences between groups in serum iron which might have resulted from the iron dextron injections. This suggests that if in fact blood loss does cause iron deficiency anemia it must be very prolonged and severe to deplete the iron reserves.

The results of the metabolic profiles from fluke infected and normal herds are shown in Table 2.

Table 2.Blood Parameters (mean values) in Fluke infected cattle and normal Herds.

	Fluke Herds	Normal Herds
Albumin	3.12	3.20 gms/dl
Globulin	4.37	4.40 gms/dl
iron	150	170 µg/dl
TIBC	367	414 µg/dl
Hemoglobin	10.6	12.0 gms/dl

The low albumin could reflect inadequate synthesis of albumin by the liver or protein loss in the biliary tract associated with fluke damage to the bile ducts. Most authors (Sewel et al. and Sinclair) conclude the anemia associated with fluke infection is due to blood loss in the gut. One would also expect the low molecular weight proteins to be lost as well which would explain the mild hypo albuminemia present. Again no evidence of iron deficiency exists in the fluke herd means nor in individual infected animals. The low albumins values in these herds could also be seen in cattle fed inadequate protein diets and makes it difficult to differentiate between the anemia of protein deficiency and fluke infection.

The study of 25 anemic and 25 control herds generated extensive data about anemia and its effects on dairy cattle. (Table 3).

Table 3.Serum iron, total iron-binding capacity and copper concentration in normal herds of cattle and herds with anemia.

Herds	iron*	TIBC*	%SAT	copper*
Fluke (5)	150	367	41%	67
Anemic (24)	160	371	45%	72
Controls (25)	170	406	42%	73

*µgm/dl

No evidence of iron or copper deficiency was found in any herd or group of cattle. The serum iron concentration usually decreases to less than 50 µg/dl while the total iron binding capacity increases to over 500 µg/dl to give a percent saturation of transferrin of 10% or less. Iron deficiency anemia does occur commonly in milk fed calves and we have recorded serum iron levels less than 25 µg/dl with a (%) saturation of 5% or less (Unpublished data). No individual values were ever recorded in that range during this study. Although copper deficiency has been reported in England, the values obtained throughout this study were above those

generally associated with copper deficiency anemia.

Evaluation of the feeding practices on each form indicated that the feeding of Brassica spp was fed irregularly or at least not uniformly from farm to farm. In those farms where it was fed Heinz bodies could be demonstrated by new methylene blue stain on erythrocytes. Thus, it was assumed that a mild hemolytic anemia was associated with the feeding of kale. Greenhalgh previously had reported mild anemias occuring as a result of Brassica ingestion.

The field study results failed to show any relationship between anemia and the interval from parturition to conception or the interval between parturition and first recorded estrum. Although anemia may influence the reproductive efficiency of cows, many other influences also exist which may mask the slight effect of a decreased hemoglobin concentration.

No statistical relationship between anemia and milk quality (milk fat; milk solids-non-fat and milk protein) was ascertained. The cows with lower hemoglobin concentrations tended to have higher milk yields within the same stage of lactation. The significance of this is difficult to explain. Previously (Payne and Kitchenham et al.) this inverse relationship was described. The critical question, does anemia limit maximum production, peak yield or milk quality remains unanswered.

Several anemic herds had low mean values for Bun , albumin and total protein. An evaluation gave evidence of an inadequate protein intake. Manston et al. have shown a chronic low protein intake will depress hemoglobin and albumin concentration whereas lowered urea-N reflected an acute decrease in protein intake. The low albumin values in fluke herds makes it difficult to differentiate between the anemia of protein deficiency and the anemia of fluke infection on a biochemical basis. Individual cows and occasionally the herd mean for globulin was increased above the norm. Many of these cows were anemic. The hypergammaglobulinemia was presumed to be associated with chronic antigenic stimulation. Chronic infection has long been associated with a mild non-responsive anemia.

This study showed that seasonal anemia in England can be associated with many causes: kale feeding, fluke infection, protein deficiency, chronic infection but not iron or copper deficiency. Most important yet to descover is the true relationship between hemoglobin concentration and yield and milk quality.

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Summary of the discussion

The sampling in most cases was between 9.00 and 11.00 a.m. in order to avoid the influence from the circadian rythm. Blood is always taken from the vena jugularis. In Switzerland a great number of cows have serum-Iron levels as low as 50-60 µg/100 ml. The reason is unknown, but it is probably not a simple iron deficiency (Blum & Zuber, Res. Vet. Sci., 1974). Haemoglobin levels of about 7 to 8 g/100 ml are not uncommon after a period of heavy kale feeding; otherwise no adverse effects are noted.

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BOVINE ABOMASAL DISPLACEMENT IN JAPAN

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Summary

Cases of the left-sided and right-sided displacements of the abomasum were encountered in Japan since 1964, mainly in dairy cows. The patients had increased annually and reached 4% of digestive disturbances in 1971, characterized by the increment of right-sided abomasal displacement. Resonant ping sounds and peritoneoscopy were useful for diagnosis. Surgical treatments were successfully performed, particularly in left abomasal displacement.

The results of the experiments carried out in goats suggest that atony of the abomasum may arise from the accumulation of unsaturated fatty acids in the rumen which are derived from diet and sent over to the duodenum without sufficient hydrogenation.

Introduction

Since 1964, numerous cases of the abomasal displacement of cattle were encountered in many parts of Japan. The overwhelming majority of the patients were then adult dairy cows suffered from the left-sided displacement of the abomasum (L.D.A.), but several cases were found in beef cattle and calves in which the right-sided displacement (R.D.A.) was predominant.

Results and Discussion

The results of our nationwide retrospective survey over a period from 1966 to 1971 on the diseases of the alimentary tract both in dairy and beef cattle show that in dairy cattle the diseases involving the alimentary tract accounted for 17 to 23% illness, and the cases with abomasal displacement increased significantly from 0.2% of digestive tract disorders in 1966 to 4% in 1971, and, finally, its rate outnumbered traumatic gastritis 2 to 1. This situation is thought to have been continuing thereafter but with a fewer increment. On the contrary, in beef cattle the occurrence of the abomasal displacement reported was very few, accounting zero in 1966 to 0.16% in 1971 at the most of diseases of the alimentary tract.

It may be noteworthy that R.D.A. showed considerable increase in those districts where the cases of the abomasal displacement augmented year by year. This was the case in dairy cows in the suburbs of large cities where lots of shoyu (soybean sauce) cake or brewer's grains were fed to the animals instead of grains or grasses, and moreover, lack of exercise was apparent. For example, in Chiba Prefecture, neighbouring Tokyo, the abomasal displacement reached 5.1% of digestive tract disorders in 1971 and R.D.A. occupied 60% of all displacements.

The situations mentioned above seem to differ considerably from those in U.S.A., Canada and European countries except Danemark, where L.D.A. outnumbers R.D.A. 8 to 1 or 20 to 1.

The resonant ping sounds revealed by percussion-ausculation over the left rib cage in L.D.A. or over the right rib cage in R.D.A. were very helpful for diagnosis, and peritoneoscopy was useful for direct recognition of the displaced abomasum to the left.

In case of L.D.A., the conservative treatment by rolling and manipulation of animals resulted in 50% recovery but in the remainder the condition recurred. Laparotomy associated with reduction of the displaced organ followed by omentopexy or abomasopexy was successfully performed in standing position or dorsal recumbency with a few relapse.

Most cases of R.D.A. were complicated with torsion. Where torsion has occurred the abomasum is not confined to one axis of rotation. It was in a clockwise or counter-clockwise direction when viewed from behind,but in some cases the abomasum and omasum have rotated in a vertical plane around a common transverse axis as described by Bischoff (1953) and Neal and Pinsent (1960). In the

latter case, prognosis was very grave.

With etiology of the disease several explanations have been presented. Dirksen (1962) proposed a multifactorial cause on L.D.A. involving mainly the interaction of late pregnancy and abomasal hypotony or atony. The most likely mediator of the relationship between the rearrangement of the abdominal viscera in late pregnancy and theabomasal displacement was based on the observations of Lagerlöf (1929). The atony was mediated by reflex nervous system or local pathways and arose from such factors as diet, stress, metabolic disturbances, and systemic or local disease (or both). Robertson (1968) expressed substantial agreement with this concept and, moreover, indicated the significance of heavy grain feeding fed in the last month of pregnancy. On the other hand, Martin (1972) described no observable association between the ration components, the method of feeding, the housing, the calving site and/or the milk production of affected cows and the occurrence of L.D.A., and advised prospective studies to further evaluate the role played by age, genetics and the number of pregnancies completed in the development of L.D.A.

In 1969 Svendsen reported that the high concentrate feeding inhibited abomasal motility by increasing the amount of volatile fatty acids (V.F.A.) which entered the abomasum. Solution containing a mixture of V.F.A. in concentrations equivalent to that of the rumen fluid from animals fed high concentrate diet also decreased therate of abomasal contractions significantly.

The authors estimated first the concentrations of V.F.A., by gas-chromatography, in specimens taken from the rumen and abomasum of goats through fistulae when ordinary diet (100 gm of the concentrate and 500 gm of hay) or high concentratediet (450 gm of concentrate and 100 gm of hay) was fed. The results obtained showed that fairly higher levels of the V.F.A. concentrations were maintained in the rumen of goats fed high concentrate diet over a period from 5 to 24 hours after feeding than those of the control,whereas in the abomasum low levels were held for 24 hours throughout in both groups fed ordinary diet or high concentrate diet. These results may have demonstrated that the high concentrations of V.F.A. in the rumen does not always imply the high V.F.A. concentrations in the abomasum.

Secondly, the motility of the pyloric part of the abomasum was examined by electromyography (EMG) after 10 ml. of the acetic, propionic or butyric acid solution (100 mM/l, pH 2.5) were infused into the abomasum of goats through fistulae. However, no observable changes were elicited in total counts of grouped discharges of each 30 minutrs, whereas the discharges shorter than 2.5 seconds of duration increased temporarily, then recovered in 90 minutes after infusion.

Then, the authors examined the effects of V.F.A., long-chain fatty acids and hydrochloric acid injected into the duodenum of goats on the motility of the pyloric part of the abomasum with EMG and strain guage mechanography. The results obtained indicated that the effects of V.F.A. (A:P:B = 6:3:1, pH 2.0, 25 ml of 50 or 100 mM/l), hydrochloric acid (50 mM/l, pH 1.2, 25 ml) and sodium stearate (1 gm) were transitional, although the concentration of the V.F.A. solutions employed were approximately 10 times as much as concentrations in normal state. On the other hand, unsaturated long-chain fatty acids (1 gm of sodium oleate, sodium linoleate and linolenic acid, respectively) showed far more distinct and durable inhibitory effects on the frequency and amplitude of abomasal contraction.

These results suggest that atony of the abomasum may arise from the accumulation of unsaturated fatty acids in the rumen which are derived from diet and sent over to the duodenum without sufficient hydrogenation.

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Summary of the discussion

The location of the electrodes and the strain gauges was defined: they were on the wall of the pyloric part of the abomasum, close to the great curvature, a few cm apart from each other. The VFA solutions contained acetate, propionate, and butyrate in the proportion 6:3:1, with pH 2.0, and 25 ml infusion volume at a concentration of 100 mM/l In the experiment with fatty acids, 1 g each of sodium stearate, sodium oleate, sodium linolate and linolenic acid was administered.

ACID-BASE DISTURBANCES IN CATTLE WITH LEFT ABOMASAL DISPLACEMENTS: RIGHT ABOMASAL DISPLACEMENT, ABOMASAL TORSION, VAGAL INDIGESTION SYNDROME, AND INTESTINAL OBSTRUCTIONS (INTUSSUSCEPTION AND CECAL VOLVULUS).

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Summary

The Acid-base and electrolyte status was evaluated in patients presented to the veterinary hospital at Cornell University with clinical signs of abomasal disease or various types of indigestion. Hypochloremic, hypokalemic metabolic alkalosis (HHMA) was present in many cases, with severe changes occurring most often in cattle with torsion of the abomasum. We were able to define a relatively unknown syndrome referred to as failure of omasal transport. In this condition, there was apparently little reflux of abomasal fluid into the rumen. The net result was dehydration and ruminal distention without alkalosis. These cows had lesions in the anterior aspect of the reticulum often located just anterior to the omasum and were usually the result of traumatic reticuloperitonitis. The prognosis in such cows was somewhat more favorable than in cows with marked metabolic alkalosis associated with impaction of the abomasum. Obstructive lesions of the small intestine (intussusception and cecal volvulus) also were associated with hypochloremic, hypokalemic metabolic alkalosis which in some cases was corrected spontaneously following surgical correction of the intestinal obstruction. The potassium-rich fluid used to treat cattle with severe metabolic alkalosis proved safe and efficacious.

Introduction

Digestive disturbances occur commonly in cattle and are often associated with clinical signs of dehydration and decreased fecal output. It was our purpose to further charaterize the acid-base and electrolyte changes associated with these disturbances for purposes of fluid therapy (if needed) and prognosis for recovery. Rumen chloride (38 cases) and blood pH, pCO_2, bicarbonate, and plasma sodium, potassium, and chloride were measured in 114 cattle with various types of digestive diseases. An intravenous fluid was designed (75 mEq/L K+; 75 mEq/L Nh_4+ and 150 mEq/L CL) to treat the most severely affected animals. Earlier studies have shown a metabolic alkalosis with many diseases of cattle (Schotman, 1971), but few relate the hypochloremia to the concentration of rumen chloride.

Results and Discussion

The plasma levels of chloride, potassium, and bicarbonate are shown in tables 1, 2, and 3.

Table 1. Plasma chloride*

	Cases	Mean	S.E.
Normal		103	-
Left Abomasal Displacement	(75)	86	±4.0
Right Abomasal Displacement	(18)	81	±3.0
Vagal Indigestion	(12)	86	±3.7
Intussusception	(5)	86	±4.4
Cecal Volvulus	(4)	91	±3.0

Expressed as mEq/L for the MEAN and Standard Error of the Mean.

Table 2. Plasma Potassium*

	Cases	Mean	S.E.
Normal		4.2	-
Left Abomasal Displacement	(75)	3.5	±0.1
Right Abomasal Displacement	(18)	2.9	±0.2
Vagal Indigestion	(12)	3.3	±0.2
Intussusception	(5)	3.3	±0.4
Cecal Volvulus	(4)	3.1	±0.2

*Expressed as mEq/L for the MEAN and Standard Error of the MEAN.

The relationship of rumen chloride to plasma chloride concentration is shown in Fig. 1. The relationship of plasma chloride to plasma bicarbonate in Fig. 2, and plasma chloride to plasma potassium in Fig. 3.

Table 3. Plasma Bicarbonate - mEq/L

		$\bar{x}$	S.E.
Normal		25	-
Left Abomasal Displacement	(63)	27	± 0.7
Right Abomasal Displacement	(18)	34	± 2.5
Vagal Indigestion	(12)	38	± 3.0
Intussusception	(5)	35	± 4.4
Cecal Volvulus	(4)	32	± 2.4

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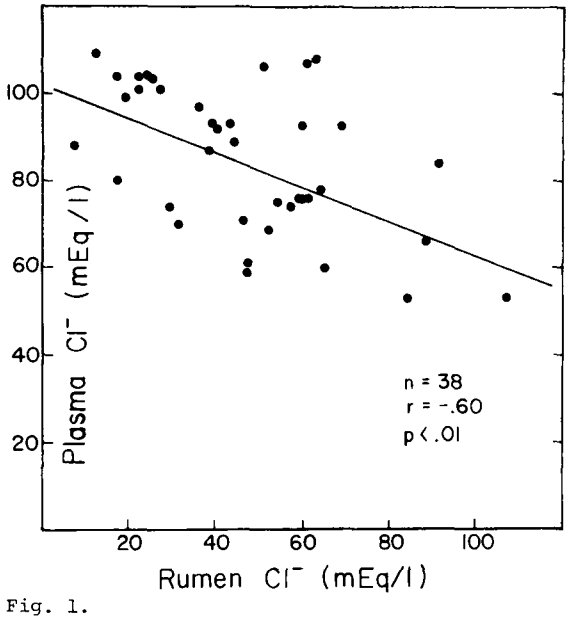

Fig. 1.

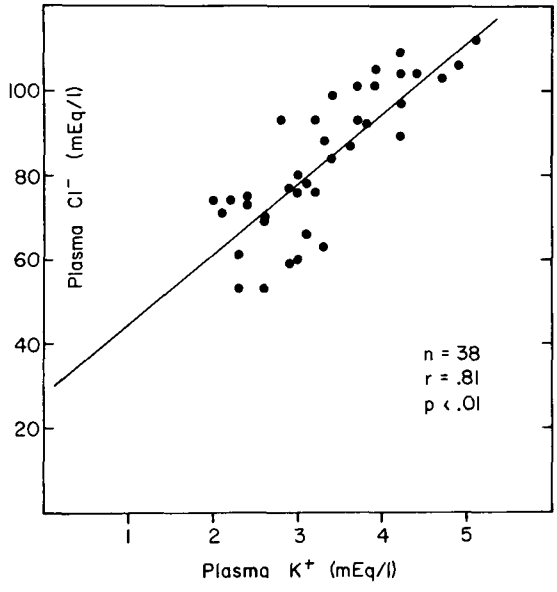

Fig. 3.

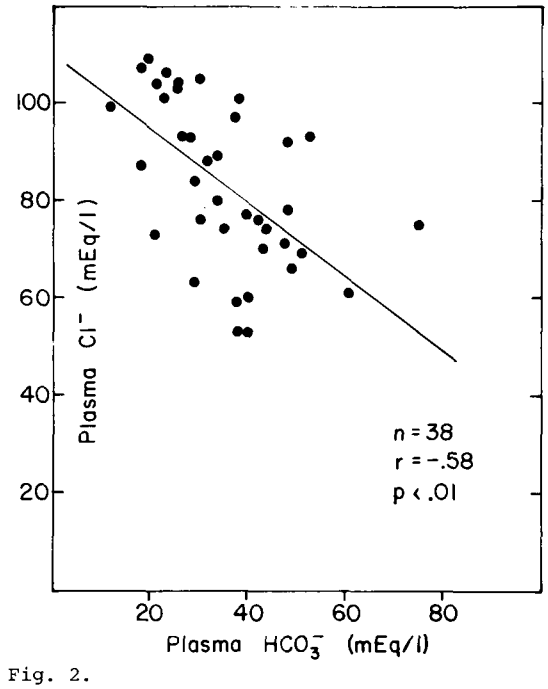

Fig. 2.

The data supports earlier work that abomasal disease and obstructive gastrointestinal lesions in cattle are usually associated with hypochloremic, hypokalemic, metabolic alkalosis. The more severe the clinical disease, the more severe the hypochloremia. The development of the hypochloremia seemed to be related to the sequestration of chloride ion in the rumen. A statistically significant negative linear correlation (r=-0.60) existed (P<0.01) between rumen chloride and plasma chloride. A similar negative correlation (r=-0.58) existed between plasma chloride and plasma bicarbonate (P<0.01). Plasma potassium was directly related (r=0.34) to plasma chloride.

This data suggests that the sequestrion of chloride in the rumen is the major perturbation associated with the development of the metabolic alkalosis. The explanations for the development of the hypokalemia are not as clearly defined but is seemingly associated with a decreased intake of potassium, continued renal excretion of potassium at levels that exceed the intake, and a shift of extracellular potassium into the cell. The lowest potassium levels were observed in cattle with the lowest plasma chloride (Fig. 3).

Thus, severely affected cattle with obstructive gastrointestinal lesions often have severe a life threatening hypochloremic hypokalemic metabolic alkalosis. Previously published data (Whitlock et al. 1975) showed that a balanced electrolyte solution rich in potassium 75 mEq/L and Nh_4 75 mEq/L with 150 mEq/L of chloride was a safe fluid when administered to normal cattle at a relatively rapid rate intravenously (5 liters/hr). It rapidly corrected the HHMA in cattle with severe alkalosis. Severely affected cattle (CL<50 mEq) without physical lesions causing obstruction were noted to improve and become normal without surgical intervention. 500 kg cattle tolerated more than 400 mEq K+ per hour intravenously without showing any cardiac irregularities or other clinical signs. The plasma potassium concentrations were monitored during the infusions and

never exceeded 5 mEq/L. It was felt that this potent alkalinizing fluid was safe and often resulted in marked clinical improvement.

During the surgical exploration of the rumen in cattle with vagal indigestion (Hoflunds Syndrome), some cattle were noted to have a relatively enlarged atonic omasum. The same cattle usually had an empty abomasum with a relatively normal plasma chloride. Since part of the mechanism of the hypochloremic alkalosis is due to reflux of abomasal secretion into the rumen, cattle with a distended rumen and a normal plasma chloride failed to transport the rumen ingesta into the abomasum. The omasum is believed to be largely responsible for this transfer. Thus, failure of omasal transport is one portion of the "Vagal Indigestion Syndrome." If the pressure in the lesion (usually an abscess between the diaphragm and omasum) could be relieved, the prognosis was often favorable for life compared to the poor-grave prognosis associated with abomasal impaction secondary to traumatic reticulitis.

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Summary of the discussion

The treatment in the case of a failure in omasal transfer is complex. 1. Supporting fluid, well balanced in electrolytes is intravenously infused. 2. A nasogastric tube is placed in the omasal canal to the abomasum and force feeding applied. 3. The abscess, if present, is drained through an incision in the reticular wall or by percutaneous needle aspiration. 4. Calcium gluconate is injected subcutaneously. The omasal lesions may be caused by the reflux of abomasal fluid that is associated with alkalosis (action of acid on the epithelium and secondary mycotic invasion will cause thrombosis of the vessels and thus ulcerations).

ABOMASAL DISPLACEMENT IN CATTLE: THE INFLUENCE OF THE RATION UPON THE COMPOSITION OF RUMINAL, ABOMASAL AND DUODENAL CONTENTS.

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Summary

The primary causative factor in abomasal displacement appeared to be atony or hypotony of the abomasum. Seemingly, high-concentrate feeding inhibits abomasal motility by increasing the amount of fatty acids which enter the abomasum. In the present study, cows with ruminal, abomasal and duodenal fistulas were fed a hay ad libitum ration or a hay and concentrate ration and were compared. It was shown that a considerable increase in ruminal volatile free fatty acid (VFFA) concentrations was not followed by a subsequent increase in abomasal VFFA concentrations. Differences in abomasal VFFA levels between the 2 rations could not be found. There was a slight, but insignificant increase in duodenal VFFA concentrations after cows were fed the hay ration. One cow, given the hay and concentrate diet, had a small, but significant increase in duodenal VFFA concentrations during the 1st. 2 hours after feeding.

The VFFA concentrations were too low to support the hypothesis that changes in duodenal VFFA concentrations could be responsible for abomasal hypotony. There were considerable differences between the rations in the sodium, potassium and chloride concentrations of ruminal, abomasal and duodenal fluid both before and after feeding.

Introduction

Most workers on the cause of abomasal displacement agree that atony or hypotony precedes displacement. Dilatation of the abomasum and accumulation of gas in the abomasum as a result of the inhibition of abomasal motility may be the cause of abomasal displacement. An increased flow of ruminal digesta to the abomasum in grain-fed cattle may be used to explain the increased amount of gas produced in the abomasum (Svendson, 1969).

Inhibition of abomasal motility can be caused by several factors, among which the nutritional factor has drawn most of the attention, although there are other possible factors (Coppock, 1974; Hull and Wess, 1973).

Nutritional disturbances as an important role in the cause of abomasal displacement are recognized since the 1st reports were published. Several authors have reported on the apparent relationship between the high concentrate, low roughage rations fed in the late postpartum period and in early lactation and the occurrence of abomasal displacement (Coppock, 1974; Pinsent et al, 1961; Robertson, 1968).

Svendson (1969) studied this relationship and showed that ruminal fluid from animals fed a high-concentrate ration significantly decreased the rate of abomasal contractions when such fluid was injected into the abomasum. The same reduction in motility was produced by injection of 300 ml of a volatile free fatty acid (VFFA) mixture in concentration equivalent to that of the ruminal fluid in grain-fed animals. Svendson concluded that high-concentrate feeding inhibited abomasal motility by increasing the amount of fatty acids which entered the abomasum.

In a subsequent study, Svendson (1970) showed that grain feeding caused increased VFFA concentrations in the abomasal digesta corresponding to a concentration that can inhibit the motility of the abomasum. Twisselman (1972), however, observed that abomasal VFFA concentrations remained relatively constant after feeding, although ruminal VFFA concentrations increased considerably. Ehrlein and Hill (1970) observed that VFFA injected into the abomasum of goats had no effect upon its motility, but abomasal activity was decreased after VFFA infusion into the duodenum.

The present experiments were done to examine the influence of the ration on ruminal, abomasal and duodenal contents of cows under circumstances that reflect the feeding routine on Dutch dairy farms. The purpose in the present report is to describe the changes in total and individual VFFA concentrations, pH and sodium, potassium and chloride concentrations.

Materials and methods

The study was performed in 4 cows with ruminal and abomasal or duodenal fistulas. All cows were given 2 experimental rations: 1st. hay ad libitum given 3 times each day and 2nd. hay and concentrates. The latter ration consisted of 5 kg of hay, 9 kg of a pelleted grain mixture, 2 kg of dried pulp and 1 kg of grass pellets. Half of this ration was fed in the morning, the other half in the afternoon. Ruminal, abomasal and duodenal contents were sampled a half hour before morning feeding and at 1 hour intervals

for 5 hours after feeding (Breukink, 1976). Samples were analysed for total and individual VFFA concentrations, pH and sodium, potassium and chloride concentrations (Breukink, 1976).

Results

Influence of diet upon ruminal and abomasal VFFA concentrations.

Mean values (+ SEM) of each series of experiments are given (Fig. 1). The data indicate that on hay ration the changes in ruminal VFFA after feeding were small. There was a rapid increase in ruminal VFFA concentration after feeding hay and concentrate. The differences in ruminal VFFA concentrations between the rations were significant. The data on abomasal VFFA indicate that there were no significant changes after feeding hay or hay and concentrate. No significant differences in abomasal VFFA concentrations between the 2 rations were found.

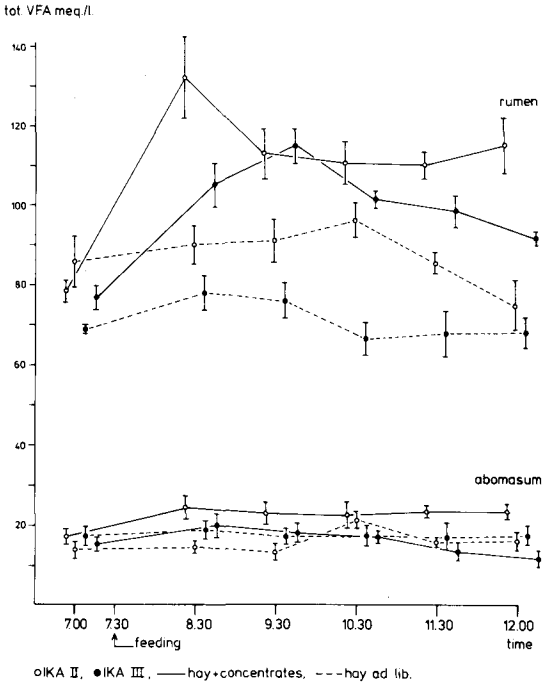

Fig. 1.

Influence of diet upon ruminal and duodenal VFFA concentrations.

Mean values of each series of experiments are given (Fig. 2). There were no significant changes in cows on hay ration and a rapid increase in ruminal VFFA after feeding hay and concentrate. Data on duodenal VFFA indicate that after feeding hay the changes in concentrations were small and not consistent. After feeding hay and concentrate a small, but significant increase in duodenal VFFA was found in 1 cow during the 1st. 2 hours after feeding.

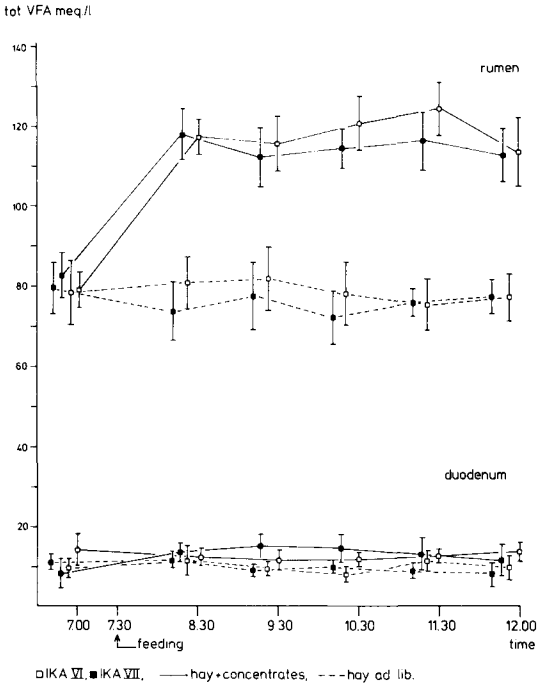

Fig. 2.

Influence of diet upon ruminal, abomasal and duodenal pH.

The changes in the pH of the ruminal fluid after feeding hay or hay and concentrate correspond with the changes in ruminal VFFA concentrations. In one cow the pH of the abomasal fluid after feeding hay and concentrate was significant higher than after feeding hay. Prefeeding values on the hay and concentrate ration were significantly higher than on the hay ration. After feeding hay and concentrate the duodenal pH decreased the first hours after feeding.

Influence of diet upon the individual fatty acids.

Mean values (+ SEM) of each series of experiments are given. The variations in the percentages are small. After the change to the hay and concentrate diet all cows showed a decreased percentage of acetic acid, no change in propionic acid and an increased percentage of butyric acid in the ruminal fluid (Fig. 3). In the abomasum and duodenum the variations are quite large. Significant differences between rations could not be found in abomasum and duodenum.

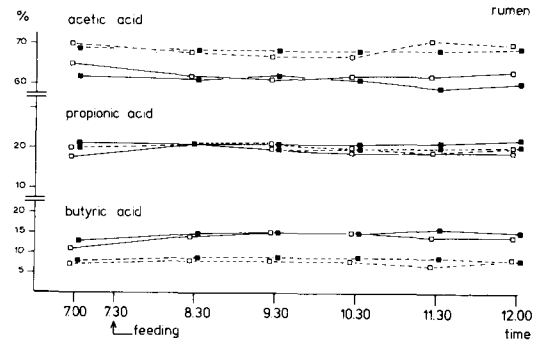

Fig. 3.
Influence of diet upon the concentrations of sodium, potassium and chloride in ruminal, abomasal and duodenal fluid.
Mean values (± SEM) of each series of experiments are given (Fig. 4I, 4II and 4III).

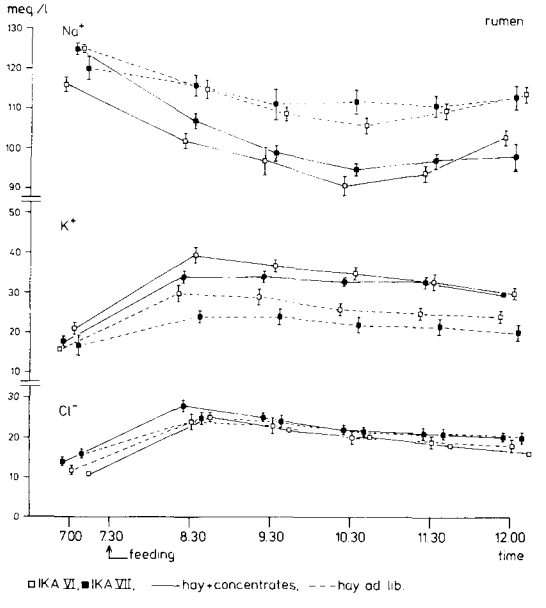

Fig. 4I.

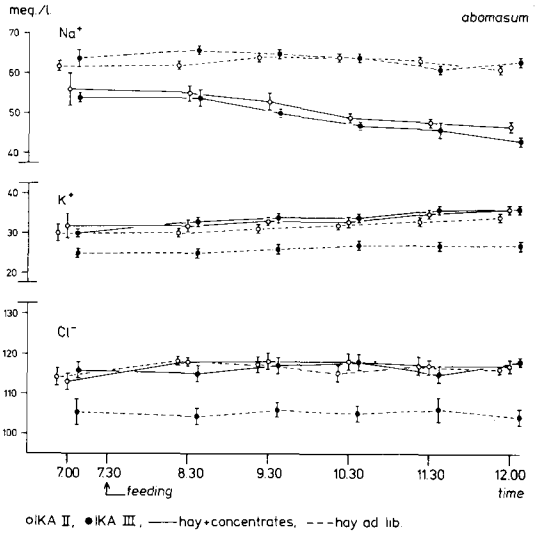

Fig. 4II.

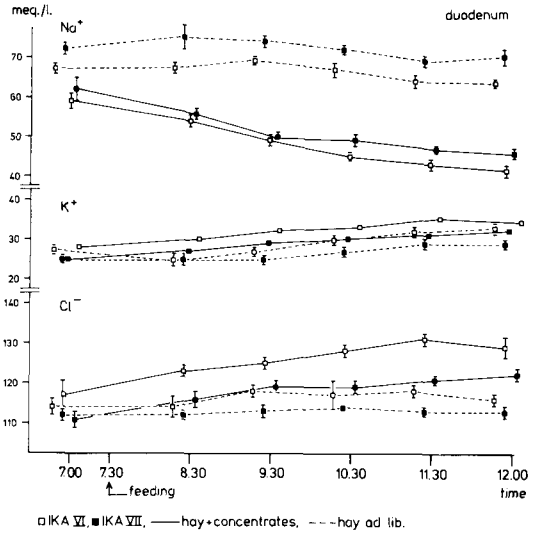

Fig. 4III.

With the morning feeding the intake of sodium, potassium and chloride was estimated. On the hay ration ± 400 meq.Na^+, ± 2000 meq.K^+ and ± 1600 meq.Cl^- was given. On the hay and concentrate ration this was ± 1000 meq.Na^+, ± 2400 meq.K^+ and ± 1500 meq.Cl^-.

Sodium.

After feeding the ruminal sodium concentration decreased on both rations. This fall was more profound on the hay and concentrate diet. After feeding no changes were seen in abomasal sodium on the hay ration. A strong decrease in abomasal sodium was seen after feeding hay and concentrate. The same picture was found in the duodenum.

Potassium.

After feeding the ruminal potassium concentrations increase shortly and decreased thereafter. The increase is the larges after feeding hay and concentrate. In the abomasum and the duodenum the changes are small.

Chloride.

After feeding both rations the ruminal chloride concentrations increased. The differences between the rations are small but significant in one cow (Ika III). The same cow showed significant differences in abomasal chloride between the rations. After feeding hay and concentrate duodenal chlori-

de levels increased to such an extend that significant differences appeared 2, 3, 4 and 5 hours after feeding.

Discussion

Results of the present study are not consonant with the observations of Svendson (1970), but confirm those of Twisselman (1973). The considerable increase in ruminal VFFA concentrations after feeding hay and concentrates was not followed by a subsequent increase in abomasal VFFA concentrations. Also, there was not any difference in abomasal VFFA levels between the 2 rations used.

In Svendson's study, abomasal fistulas were situated in the pyloric part of the abomasum.

In the present experiment, the increase of VFFA concentrations in the rumen after feeding concentrates persisted for several hours. That means that any effect or ruminal VFFA on abomasal VFFA should have been seen during the experimental period.

Svendson (1969) suggested that high concentrate feeding increases the flow of ingesta into the abomasum.

Omasal fluid is diluted immediately after entering the abomasum. The dilution depends on the amounts of abomasal juice secreted. An increase of VFFA concentration in abomasal affluent increases the gastric secretion, thus maintaining the concentrations of VFFA in the abomasal fluid within relatively narrow limits (Ash, 1961).

In the present study, the mean abomasal VFFA concentrations in cows on the hay ration ranged from approximately 9 mEq/L to 19 mEq/L. This is rather high compared with the values given by Svendson, who had found earlier that a VFFA concentration of 10 mEq/L in the abomasal content produced an inhibition of abomasal motility. Although fistulas in Svendons's work were situated in the pyloric region, it is unlikely that this can explain the differences.

In cows with the duodenal fistulas in the present study, it was found that in the cows fed hay ration, duodenal VFFA still were between 8 mEq/L and 11 mEq/L. The VFFA concentrations in duodenal fluid in cows given hay and concentrates were between 8 mEq/L and 15 mEq/L and thus were only slightly lower than the concentrations found in the abomasum. To obtain only a slight reduction of motility of the abomasum, Ehrlein and Hill (1970) needed a 100 to 150 mM, acetic, propionic, or butyric acid solution/L.

Possibly, the VFFA absorption in the fundic region is of such a magnitude that most of the VFFA is absorbed by the time the ingesta reaches the fistulated area. The local effect on motility may then be limited to the fundus and the cranial part of the body of the abomasum, where motor activity of the abomasal wall is low (Benzie and Phillipson, 1957).

Results of the present experiment do not support Svendson's explanation for the occurrence of abomasal displacement in cows fed high concentrate, low roughage rations. Also, it could not be proved that changes in duodenal VFFA concentration might be responsible for abomasal hypotony.

The results of the changes of the individual fatty acids did not reveal unexpected facts. The increased percentage of butyric acid is probably due to the high amount of beetpulp used. The strong variations in the percentages of the fatty acids in abomasum and duodenum hampers conclusions.

The rapid decrease in sodiumconcentration despite higher intake is due to the rapid increase in ruminal osmolarity and potassium concentrations. Both enharce sodium absorption in the rumen and water secretion. Ruminal chloride levels are under strong influence of the saline chloride.

The fate of the sodium, potassium and chloride depend upon the concentrations and flow of the affluent, the secretion in the abomasum, the absorption in the abomasum and the passage time through the abomasum. The concentrations in the duodenum are almost the concentrations of the chyme leaving abomasum, because the duodenal fistulas were situated $\pm$ 10 cm from the pylorus. It is likely that most of the changes are due to changes that occur in the omasum (Engelhardt and Hauffe, 1975), except for chloride, that is actively secreted in the abomasum.

The fact that after feeding no significant changes occur in the chloride concentration of the abomasum, indicates that secretion is more or less constant.

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Summary of the discussion

The hay diet consisted of 12-15 kg fed at 7 a.m., 3 p.m. and just before night (leftovers were removed). The mixed diet contained 5 kg of hay, 9 kg of concentrates, 2 kg of dry pulp (pelleted) and 1 kg of grass (pelleted). In the rumen fluid no lactic acid was found. Volatile fatty acids had a local effect in the abomasum, no inhibition was seen of the rumen motility unless at very low pH. Grass and hay were of normal quality, although the crude fiber content of 35 seemed a little high. The location of the fistulae seems to be very important; ours were in between the pyloric and fundic areas used by other investigators. Despite the increased sodium intake in the concentrate ration, the rapid fermentation resulted in increased osmolarity with higher potassium and lower sodium contents. The flow of saliva would later normalize these values.

OBSERVATIONS ON EXPERIMENTALLY INDUCED GIZZARD EROSION IN THE DOMESTIC FOWL (GALLUS DOMESTICUS L.)

HISTOCHEMICAL AND (SCANNING) ELECTRONMICROSCOPIC OBSERVATIONS ON THE GIZZARD LINING (GLYCOCALYX)

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Introduction

The term glycocalyx or cell coat is used for the macromolecular layer covering the outer cell surface. Secretions of other cells or glands may adhere to the glycocalyx or mix up with it. The glycocalyx has many functions. Among these control of transport into and out of the cell and determination of the cell's social behaviour in recognition and cellfusion are of great importance.

The glycocalyx of the gastro-intestinal canal is thought to be the site of the gastro-intestinal barrier, protecting the vulnerable mucosa against attack by aggressive intestinal contents. In the fowl gizzard the lining or koilin layer may fulfill this glycocalyx function. Failure on this point is known as gizzard erosion.

The disease may occur in varying degrees of severeness, from small focal submucosal bleedings to occasionally perforating ulcerations. Gizzard erosion shows at least analogies with gastro-intestinal bleeding and ulceration in other animals and men.

Janssen devised experimental rations that consistently produce gizzard erosion. In contrast with the gastro-intestinal glycocalyx in general the gizzard glycocalyx can easily be isolated. In vitro experiments are planned to examine its barrier function. We hope to find parameters for expression of its protective action so that it may be used as an in-vitro glycocalyx model. That might enable us to study the fundamentals of the barrier function and to test aggressive agents avoiding cumbersome feeding experiments.

In preparation of such experiments we made observations on formation, structure and composition of the gizzard glycocalyx, using histochemical reactions,(scanning) electronmicroscopy and chemical analysis.

Formation

The gizzard glycocalyx is formed by branched, tubular mucosal glands, arranged in groups. The gland-base secretes vesicles with granular contents, fusing into "rods". Some empty glandbases may be seen with cells in a resting state. In the body of the glands vesicles are extruded that break up setting free their lacy contents to form a mantle round one or more rods. Both secretions take on a fibrillar appearance by sticking together or/and by streaming effects in the viscous material in passing through the glandtube. Toner (1964) does not differentiate between these two types of secretions. The neck or topcells are the site of production of sialomucin, which in higher quantities is known to diminish the cohesive properties of the glycocalyx. Their secretion impregnates the secretion of the gland bodies giving coherence to the glycocalyx. The three types of secretion might be three phases in the gland cell's life, during which the cell moves upwards along the tubule to be periodically shedded at the mucosal surface. Then it is incorporated in the glycocalyx.

Structure

The gizzard glycocalyx covers the mucosal surface as an apparently hyaline layer. Normally it is not scaly, scaliness in sections being a cutting artefact. Its surface is uneven, the ends of the rods protruding over it. These may be secreted at a higher rate or may be tougher than the rest. This serves the grinding function. Dried and broken its fracture looks glassy like flintstone. During its secretion the glycocalyx solidifies. Torn from the mucosal epithelium the underside looks velvety, a great part of the rods, already solidified and one with the glycocalyx being drawn out of the tubules. According to Webb and Colvin (1964) solidification is caused by gastric HCl. The nature of the secretion process makes the glycocalyx inhomogeneous in tangential as well as in transverse direction. Only tangential slicing will be of use for serial analysis.

Composition

Histochemical procedures showed the glycocalyx having a glycoprotein nature. Rods as well as topcell secretion contain carbohydrate components.Only the topcell produces sialomucins.

Aminoacid analysis gave results comparable with those of Webb and Colvin (1964). Comparing normal and severely eroded glycocalyx material a tendency was found for decreasing contents of aminoacids in the dry matter. Lysine and histidine form an exception, they tend to increase. Dry matter content is much higher in the normal than in the eroded glycocalyx, 54 vs. 36%. Preliminary data for sugar content amount to 2-4% in the dry matter, being higher in erosion. In case of erosion the ratio of aminosugar to neutral sugar was lower and that of sialic acid to neutral sugar higher. The neutral sugars are mainly galactose and glucose with traces of fucose and mannose. The eroded glycocalyx had relatively more galactose. As sialic acid is known to decrease glycocalyx fusion, the higher ratio may explain the loose, flocculous texture in erosion. The excessive shedding of topcells in erosion might be the origin of the higher sialic acid ratio. Erosion may develop,as well as heal,in a few days.

Acknowledgements

Thanks are due to Dr. B.D.E. Gaillard, Lab. Anim. Physiol., Wageningen, for her help in carbohydrate analysis and to Mr. S. Henstra, TFDL, Wageningen, for TEM and SEM fotografy.

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Summary of the discussion

The amino acid composition of the glycocalix was the same as reported by Webb and Colvin. The dry matter content of normal gizzard is approximately 54%, of eroded gizzards only 36%. The sugar content is 2-4% d.m., and in the eroded gizzards the glycocalix contained more sialic acid than in the healthy ones.

NUTRITIONAL RESEARCH ON THE FACTOR(S) CAUSING GIZZARD EROSION

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Summary

Preliminary experiments had shown that Peruvian anchovy fishmeal can cause gizzard erosion. In 5 further trials with Corny Rock x White Plymouth Rock broilers, heating of non-toxic South African and Canadian fishmeals produced a toxic factor that caused gizzard erosion. Separation of a toxic fishmeal into three fractions of particle size fine, middle and coarse and inclusion of these separate fractions in experimental feeds proved that the toxic factor was concentrated in the fine fraction. The finer the fraction, the more severe the gizzard erosion. The histamine content of these fractions was not related to severity.

Introduction

Some years ago Janssen (1970 and 1971) found that Peruvian anchovy fishmeal could be a cause of gizzard erosion. The higher the level of fishmeal in the ration for broilers, the more severe the erosion.

Another conclusion from the first experiments was that the fishmeals were quite variable in their toxicity. Sometimes moderate levels caused a large incidence of severe erosion with high mortality. Studying the carcasses of the dead fowl often perforation of crop, oesophagus, gizzard and duodenum was observed, and the gut was filled with a black or darkbrown material, probably discoloured blood.

This paper discusses some experiments on the effects of overheating and sieving the fishmeal in fractions.

Experimental feeds, birds and the erosion score

The composition of the feed will not be described in detail. For groups with fishmeal or fractions of it, the same rations were used as described in 1971. The birds were broilers from a commercial cross Corny Rock x White Plymouth Rock. Most trials lasted 7 weeks. The judgement of the gizzard erosion was given in the form of a score. Healthy gizzards scored 0 or 1. Clearly eroded gizzards scored 2 and gizzards severely eroded, often with haemorrhage in the linings, scored 3. An aggregate score for a group was calculated as follows:

$$\text{Aggregate score} = \frac{\text{number with score 2} \times 2 + \text{number with score 3} \times 3}{\text{number of gizzard examined}} \times 100$$

The mortality due to gizzard erosion was not included in the score.

Trial 1

From literature we have got indications that artificially dried maize sometimes could cause gizzard erosion. In Trial 1 maize, wheat, barley, oats and sorghum were soaked in water and after that artificially dried for 4 hours, with a moderate temperature of 80°C*. The treated grain was included in the experimental rations up to 50%. No difference from untreated grain was observed in the occurrence of gizzard erosion. A South African fishmeal, known not to be toxic from a preliminary trial, even when 25% was mixed in the ration, was heat-treated** for 2 days (8 hours per day). The temperature varied between 145°C and 160°C. The treated fishmeal formed 25% of the ration.

Results

Locally the gizzard linings of this group were eroded, but very slightly. Feed-consumption was abnormally low and as a consequence growth was retarded.

Trial 2

It was decided to repeat the trial with a lower proportion of heat fishmeal (15%).

Discussing the problems concerning the identity of the factors causing gizzard erosion, the possibility was put forward that the coarse, hard particles, for instance originating of crawfish and lobster, could be the cause. In this experiment this hypothesis was tested.

For this part of the trial a toxic fishmeal of Peruvian origine was sieved into three fractions: fine, middle and coarse. The sieves used were 30 mesh and 60 mesh. They divided the fishmeal into almost equal parts. The level of the fishmeal and of the fractions in the rations was 15%.

The results are given in Table 1.

* at TNO CTI, Delft
** at Instituut voor visserijprodukten TNO, IJmuiden

Table 1. Gizzard erosion score

	Score	
	Males	Females
15% heated fishmeal*	57	51
15% coarse fraction**	5	17
15% middle fraction**	89	72
15% fine fraction**	150	172

*South African

**Peruvian

A striking effect of the sieving is demonstrated in this table. In contrary to what was expected the factor was concentrated in the fine fraction and not in the coarse one.

The heated fishmeal produced a rather severe gizzard erosion.

In the group with the heated fishmeal and the middle fraction, there was no mortality due to gizzard erosion. In the group with the coarse fraction, one fowl (1%) died with gizzard perforation. In the fine-fraction-group 8% of the fowl died with perforations in the crop and gizzards; in 2% gizzard erosion without perforation was detected.

For this trial a sample maize, wheat and sorghum were also heattreated. The drying temperature was 120°C and the duration of the treatment 4 or 18 hours.

The results show, that these heattreatments did not induce gizzard erosion in the experimental groups.

Trial 3

Another batch of peruvian fishmeal was sieved and the fine and coarse fractions were compared with the complete fishmeal.

Results are given in Table 2.

% in the ration	Kind of fishmeal	Score males	females
25%	peruvian fishmeal	150	174
15%	coarse fraction	22	8
15%	fine fraction	128	116

From these data can be concluded that a ration with 15% of the coarse fraction hardly gave gizzard erosion, while 15% of the fine fraction produced erosions with a severity almost comparable with the effect of 25% of the complete fishmeal.

In the group with 25% fishmeal, 5 fowl died with perforations. In the group with the fine fraction, one fowl died with a perforation.

Trial 4

The hypothesis was developed that the erosion factor was the result of overheating and as a consequence was concentrated in the fine fraction. This hypothesis could be tested by over-heating the complete fishmeal (thus also the coarse fraction). This was done in Trial 4. The overheated fishmeal was sieved into three fractions.

Unfortunately the effect of the heattreatment was not so pronounced as in Trial 2. Even the scoring method had to be changed. With this new system, the groups with the coarse, middle and fine fractions, respectively, got score 28, 42 and 77. This means that the incidence of small local aberations increased when the fractions became finer. Obviously even under these circumstances, the factor seems to be concentrated in the fines of the fishmeal.

Trial 5

In the fifth and last trial, another peruvian fishmeal was separated into fine, middle and coarse fractions; and a fishmeal, this time a Canadian meal, was heattreated. The temperature was in the range of 140 to 160°C. The duration of the heattreatment was 16 hours. The results are given below.

Table 3. Erosion score

% in the ration	Kind of fishmeal	total score
25%	Canadian	8
15%	Heated Canadian	53
15%	fine fraction Peruvian	165
15%	middle fraction Peruvian	88
15%	coarse fraction Peruvian	30
	Control ration	10

This table proves that also in the Canadian fishmeal, the gizzard factor can be induced by heattreatment. The scores of the three fractions confirm the results of the other experiments. In all trials, there were no consistent differents in crude protein, fat and ash content of the three fractions.

Harry et al. (1975) related histamine to gizzard erosion. The concentration of the gizzard factor in the fines of the fishmeal offered the opportunity of testing the role of histamine in the fishmeal. Fisher (1976) determined the histamine content in the fractions used in Trial 5. The analytical data demonstrated no relation between

histamine content and incidence of gizzard erosion.

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Summary of the discussion

The gizzard erosion of one day old chicks and the erosion induced by copper sulphate in older birds are different phenomenons. At least the morphological effects are different, the histological consequences of copper sulphate feeding have not been studied yet. In the non-fed young chicks the symptoms must be related to the dramatic change from external to internal blood supply during the last day of the hatching period; maybe the suddenly increased blood pressure is a causing factor.

ABOMASAL MUCOSAL BLOODFLOW IN RELATION TO ABOMASAL SECRETORY ACTIVITY IN SHEEP

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Introduction

Ration composition has been suggested as an important factor in the etiology of abomasal bleeding in adult cattle (Aukema and Breukink, 1974). So far, it has not been established which agents are responsible for the injury of the abomasal mucosa, the formation of abomasal ulcers and subsequent abomasal bleedings.

Damage of the mucus layer, which protects the abomasal mucosa, probably by interference with mucus synthesis, is possibly caused by agents present in the ration or produced during the fermentation process in the forestomachs. Injury of the mucus layer will cause a high flux of hydrogen ions into the mucosa. The release of histamine is increased and acid secretion and mucosal bloodflow are stimulated, as has been pointed out by Davenport (1972) for non-ruminants.

Such a positive relationship between acid secretion and mucosal bloodflow has also been frequently suggested, when the protecting mucus layer is not damaged. Gastric acid secretion is an active process requiring energy and thus substrates. Therefore it has been suggested that mucosal bloodflow is related to gastric acid secretory activity. After various pharmacological as well as physiological gastric acid secretory stimuli, evidence has been provided both for and against such a relationship (Jacobson, 1967).

In our experiments sheep abomasal acid secretion was stimulated by intra-abomasal infusions of soyaprotein and $KHCO_3$. Abomasal mucosal bloodflow was estimated simultaneously by the aminopyrine clearance method (Jacobson et al., 1966).

Methods

Experimental animals

The experiments were carried out on three Texel wethers, weighing 45-60 kg. Re-entrant cannulas were fitted into the proximal part of the duodenum, a few centimeters behind the pylorus (Hogan and Phillipson, 1960). The experimental substances were introduced into the abomasum through a silicone infusion tube, inserted into the abomasal fundus. Sheep were kept individually in metabolism cages. Rations consisted of hay (600 g/day) and concentrates (300 g/day), supplied in six equal portions every four hour period.

Experimental lay out

Both abomasal infusates, a 10% soyaprotein suspension in physiological saline and a 0.30 M $KHCO_3$ solution, were introduced into the abomasum of the sheep comparatively to a control (no intra-abomasal infusion) according a 3 x 3 Latin square design. Infusions were carried out continuously, starting about 40 hours in advance of the first experimental period.

About sixteen hours before the first experimental period, catheters were inserted into both jugular veins. Through one of these catheters a 0.5% solution of aminopyrine in physiological saline was infused at a rate of about 20 g/h. The other catheter, which was used to draw blood samples, was kept open by infusion of physiological saline at a comparable infusion rate.

During each intra-abomasal infusion, duodenal digesta were collected for eight hour periods (8.00 h - 16.00 h) on three consecutive days. The duodenal re-entrant cannulas were disconnected and digesta leaving the proximal cannula were allowed to flow into a vial kept at body temperature. After each period of ten minutes vial contents were weighed, a 10% sample was removed and the remainder was returned into the duodenum through the distal cannula. Samples were pooled for each experimental period of eight hours. The pooled samples were stored at -20°C.

Simultaneously every hour a jugular blood sample was drawn, starting at 7.30 h. Blood samples were heparinized and the supernatant plasma (Christ centrifuge,2000 g) was pooled and stored at -20°C for each experimental period.

Analysis of intra-abomasal infusates

Samples were titrated to pH 3 with 0.1 N HCl for the estimation of the in vitro buffering capacity (BC). Dry matter content (DM) or the soyaprotein suspension was determined by drying to constant weight at 101°C. Of the $KHCO_3$ solution dry matter content was calculated based on its molar concentration. Nitrogen was estimated by the Kjeldahl method, crude protein (CP) being calculated as N x 6.25. Analysis of chloride (Cl) was performed on the supernatant solution (Christ centrifuge, 2000 g) according to the

method of Volhard. Osmotic pressures (OP) were estimated in the supernatant solutions (MSE 65 ultracentrifuge, 70000 g) with the Knauer milliosmometer (depression of freezing point). Infusion rates of in vitro buffering capacity (IRBC), dry matter (IRDM), crude protein (IRCP), and chloride (IRCl) were calculated by multiplying the concentration by the individual infusion rates (IR).

Analysis of duodenal digesta

Duodenal passage rates of digesta (PRD) were measured during the experiments. The average (g/h) was calculated for each experimental period of eight hours. In the duodenal samples dry matter (DM), crude protein(CP), chloride (Cl), and osmotic pressure (OP) were estimated as described under intra-abomasal infusates. After measuring the pH, total acid concentration (TA) was determined by titration to pH 7 with 0.1 N NaOH. Duodenal passage rates of dry matter (PRDM), total acid (PRTA), crude protein (PRCP), and chloride (PRCl) were calculated by multiplying the concentration by the individual duodenal passage rates of digesta.

Abomasal mucosal bloodflow

Aminopyrine concentrations in plasma and duodenal supernatant (MSE 65 ultracentrifuge, 70000 g) were estimated according to the method of Brodie and Axelrod (1950). The abomasal mucosal bloodflow (AMBF) was calculated by dividing the amount of aminopyrine passing the proximal duodenum by plasma aminopyrine concentration.

Statistics

Data were analysed statistically according the analysis of variance as reported by Snedecor (1962) for a Latin square design. Statistical comparisons of the duodenal data after both intra-abomasal infusions versus the control duodenal data were made using the two-sided Student t test (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$).

Results

Data on the intra-abomasal infusions of the 10% soyaprotein suspension in physiological saline and of the 0.30 M $KHCO_3$ solution are given in table 1. The effect of both intra-abomasal infusates on duodenal characteristics and thus on abomasal secretory activity is shown in table 2.

Table 1. Characteristics of the intra-abomasal infusion of 10% soyaprotein and 0.30 M $KHCO_3$. (For abbreviations see methods)

		Soyaprotein	$KHCO_3$	SE
IR	g/h	64.9	66.9	0.5
IRBC	meq/h	9.88	20.52	0.12
IRDM	g/h	6.39	2.01	0.04
IRCP	g/h	5.48		0.03
IRCl	meq/h	7.76		0.07
OP	mosm/kg	361.7	510.3	0.6

Duodenal passage rate of digesta was increased after both intra-abomasal infusions. The increases were higher than the amounts infused, which is in accordance with the finding that abomasal secretion was stimulated by both intra-abomasal infusates.

After intra-abomasal infusion of soyaprotein duodenal passage rates of dry matter and crude protein were significantly increased. Both increases did not differ significantly from the amounts infused, indicating that the control duodenal passage rate of digesta and thus the abomasal digesta entering rate is not affected by the continuous infusion of soyaprotein. The same was found after intra-abomasal infusion of 0.30 M $KHCO_3$. This shows that the stimulative effect of the intra-abomasal infusates on abomasal acid secretion can be determined by comparing the duodenal passage rates of total acid and of chloride after both intra-abomasal infusates with the amounts passing the duodenum when no intra-abomasal infusion is carried out.

The pH of digesta entering the abomasum approximates neutrality. Thus titration of the acid duodenal contents to pH 7 can be regarded as a good approximation of the amount of acid secreted in the abomasum. This takes not into account the back diffusion of hydrogen ions from the abomasal contents to the bloodstream. Davenport (1967), using unstimulated, vagally denervated fundic pouches in dogs, found H^+ losses of 3-14% after 30 minutes. Also a minor neutralization of the acid digesta leaving the abomasum may have been caused by the alkaline juice secreted by the Brunner glands situated proximally to the re-entrant cannula. This effect seems to be of little importance since in sheep no differences in acidity were found between digesta collected from an antral cannula and from a cannula inserted into the proximal duodenum (Weston, 1976).

After intra-abomasal infusion of soyaprotein the increase in the duodenal passage rate of total acid equalled the in vitro buffering capacity infused.

Table 2. Effect of intra-abomasal infusion of a 10% soyaprotein suspension and of 0.30 M $KHCO_3$ on abomasal acid secretion and abomasal mucosal bloodflow as determined in the proximal duodenum. (For abbreviations and significances (*) see methods)

		Control	Soyaprotein	$KHCO_3$	SE
PRD	g/h	559.1	656.7 **	697.3 ***	14.8
PRDM	g/h	19.77	26.50***	21.14	0.53
PRTA	meq/h	31.54	39.69***	30.29	0.91
1	meq/h			41.48***	0.94
pH		2.82	2.99***	3.13***	0.02
PRCP	g/h	5.30	10.84***	5.16	0.14
PRCl	meq/h	63.80	81.15***	73.57**	1.50
2	meq/h		73.39**		
OP	mosm/kg	245.6	267.9 ***	239.4 **	1.0
AMBF	l/h	11.17	9.87	13.37	1.03

1 PRTA corrected for capture of hydrogen ions by bicarbonate ions

2 PRCl corrected for IRCl

However, a significant increase in the duodenal pH was found, suggesting that the buffering capacity of the soyaprotein infused was increased, probably by a partial peptic hydrolysis of the protein. After correction of the duodenal passage rate of chloride for the amount of chloride infused, the increase of the duodenal passage rate of chloride after soyaprotein infusion, compared with the increase in the duodenal passage rate of total acid, indicates that hydrogen and chloride ions are secreted in equimolar amounts in the abomasal juice secreted extra.

After intra-abomasal infusion of 0.30 M $KHCO_3$ the duodenal passage rate of total acid was not found to be increased. Introduction of bicarbonate ions into the abomasum causes a production of carbondioxide, resulting in a loss of hydrogen ions and thus in a decrease in total acid concentration. Due to this carbondioxide formation, ions disappear from the abomasal contents and thus the abomasal and duodenal osmotic pressure will decrease too. Although a hypertonic $KHCO_3$ solution was infused into the abomasum, duodenal osmotic pressure was found to be even lower than the duodenal osmotic pressures in the control experiments. Using the average duodenal osmotic pressure of the control as a reference, the theoretical duodenal osmotic pressure for each experimental period was calculated, assuming that no hydrogen ions had been captured by bicarbonate ions. Based on these calculated osmotic pressures the duodenal passage rates of total acid after intra-abomasal infusion of $KHCO_3$ were increased by half of the milliosmoles lost. This is because the decrease of the osmotic pressure is caused by equimolar amounts of hydrogen and bicarbonate ions. After this correction the increase in the duodenal passage rate of total acid after intra-abomasal infusion of $KHCO_3$ approximated the increase in the duodenal passage rate of chloride. This also indicates that the additional hydrogen and chloride ions are secreted in equimolar amounts.

In dogs Jacobson et al. (1966) found ratios of up to 40: 1 between the concentration of aminopyrine in gastric juice and in plasma during active gastric secretion. Based on the pH difference between gastric contents and plasma a ratio up to 10000:1 was expected. That the ratio found was much lower than the theoretical equilibrium ratio suggests that the rate at which aminopyrine is delivered to the membrane for transport is probably determined by the gastric mucosal circulation. This finding indicates that the clearance of aminopyrine through the gastric mucosal membrane can be regarded as a reliable approximation of gastric mucosal bloodflow. Curwain (1972) has shown equilibration of aminopyrine between erythrocyte cytoplasm and physiologica saline to be complete within 1 minute. This suggests that aminopyrine not only in plasma but also in the blood cells will be cleared almost quantitatively. In our experiments we found ratio's up to 20:1 between duodenal digesta and plasma aminopyrine concentration. Lower values than the ratio, cited above, were expected, as the abomasal juice is diluted by the abomasal contents.

Based on the duodenal passage rates of total acid, both of the intra-abomasal infusates stimulated abomasal acid secretion by about 30%. This secretory response was not paralleled by an increase in abomasal mucosal bloodflow.

Discussion

Addition of buffering compounds to the abomasal contents probably acts on abomasal acid secretion through an increase in the pH of the abomasal contents. Different hormones are probably involved in the regulation of abomasal acid secretion. One of these, gastrin, has been identified in abomasal mucosal cells (Andersson et al., 1962), especially in the abomasal antrum. Gastrin stimulates abomasal acid secretion after release into the bloodstream. The release of gastrin is inhibited at a lower intra-abomasal pH, as has been shown in the stomachs of non-ruminants (Andersson and Elwin, 1971). This effect of the pH on gastrin release might explain the effect of the $KHCO_3$ buffer on abomasal acid secretion.

Protein break-down products are known as active gastrin releasers. Thus soyaprotein could be expected to stimulate abomasal acid secretion more actively than $KHCO_3$. Relative to the in vitro buffering capacity infused, intra-abomasal infusion of soyaprotein was found indeed to be a more active stimulator of abomasal acid secretion.

In pure abomasal juice chloride ion concentration has been found to exceed hydrogen ion concentration (Hill, 1968). When abomasal secretory activity was higher, however, this ratio was found to decrease. Thus a stimulated abomasal secretory activity is not effectuated only by an increased secretion rate but also by a higher concentration of especially hydrogen ions in the abomasal juice. This probably explains the 1:1 ratio which we found between the additionally secreted hydrogen and chloride ions.

Gastric acid secretion and gastric mucosal bloodflow have been shown to increase simultaneously after various gastric secretory stimuli. Jacobson and Chang (1969) found an increased gastric mucosal bloodflow in conscious dogs after stimulation of gastric acid secretion at comparable rates by intravenous infusion of histamine and porcine gastrin. After histamine, however, a significantly higher mucosal bloodflow was observed, indicating that histamine exhibits more vasodilator activities and that gastric acid secretory activity and gastric mucosal bloodflow are not necessarily related to each other directly. This finding has been confirmed by Domanig et al. (1966) who showed gastric acid secretory activity after a prolonged intravenous histamine infusion in dogs to be stimulated in excess of gastric mucosal bloodflow, resulting in a decreased gastric venous oxygen saturation. In our experiments a positive relationship between abomasal mucosal bloodflow and abomasal acid secretory activity could not be demonstrated either. These results, together with the deviations of a constant ratio between gastric secretion and gastric mucosal bloodflow, cited above, provide new arguments that secretory activity and mucosal bloodflow are not related directly.

Abomasal or gastric mucosal injury was accompanied by a stimulation of mucosal bloodflow (Davenport, 1972). A damaged abomasal mucosa facilitates back-diffusion of hydrogen ions of the abomasal contents into the bloodstream. No indications of such effects have been found in our experiments. Based on this evidence it seems unlikely that injury of the abomasal mucosa is caused by stimulation of abomasal secretory activity during short term periods. This finding, however, does not exclude the possibility that abomasal mucosal injury may be related to abomasal secretory activity after a more prolonged period of stimulation.

Acknowledgement

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Summary of the discussion

Effects of various dietary regimes and drugs have not been studied systematically yet. It is clear that the increase of protein content of diets stimulate abomasal acid secretion, and so did the addition of buffering compounds to the diet. A highly significant relationship was observed between the duodenal passage rates of crude protein and of total acid. Intra-abomasal infusion with $KHCO_3$ decreased the duodenal osmotic pressure (0.15 M even more so than 0.30 M). Differences in osmotic pressure are not expected to influence the clearance rate of amino pyrine, although for the transport by the abomasal mucosa a direct relation exists with the gradient of the acidity between plasma and abomasal contents.

RUMEN ACIDOSIS AND METABOLIC KINETICS OF D(-)LACTIC ACID

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Summary

The paper considers conditions of increased rumen production of D-lactate and its elimination from the blood after absorption. Data on the biokinetics of D-lactate, renal excretion, oxidation to CO_2 including tissue slices of heart, kidney, liver and rumen epithelium, and conversion to glucose represent a new basis for the understanding of isomer metabolism and of D-lactacidosis.

Introduction

Rumen acidosis is a well-known consequence of overfeeding with fiber-deficient diets. Other factors as sulfur deficiency (Whanger & Matrone, 1966) may also play a role. The clinical aspects of the disease have been outlined (Dirksen 1970; Dunlop 1970; Dunlop & Hammond 1965). A severe imbalance of the rumen production and utilization of lactic acid appears to be the central problem. Paramount importance has been ascribed to D(-)lactic acid but the animal biochemistry of this unphysiological isomer has remained obscure.

Results and discussion

Rumen

D-lactate is a normal rumen intermediate which is produced together with L-lactate within 15-30 min after ingestion of carbohydrates and eliminated by lactilytic bacteria at a rate of about 100%/h. On changing over to lowfiber diets lactate is produced faster than ulilized. If the acidity can be maintained about pH 5 for 2-5 days and sufficient aminoacids are present, lactilytic bacteria, in particular Megasphera (Peptostreptococcus) elsdenii, will increase in number and eventually bring pH to about 5.2 - 5.7 — a status of adaption with latent (or compensated) acidosis (Giesecke & Bartelmus, 1972; Giesecke & Geiges, 1974; Giesecke, Bartelmus & Stangassinger, 1976; Ogimoto & Giesecke 1974). If adaptation fails lactic acid will become predominant and rise exponentially to 15-20 g/l lowering pH to values less than pH 5 (Fig 1). In this range the proportion of D-lactate is increased to 40-50% becoming a potential problem after absorption, which may be faster than for L-lactate (Williams & Mackenzie, 1965).

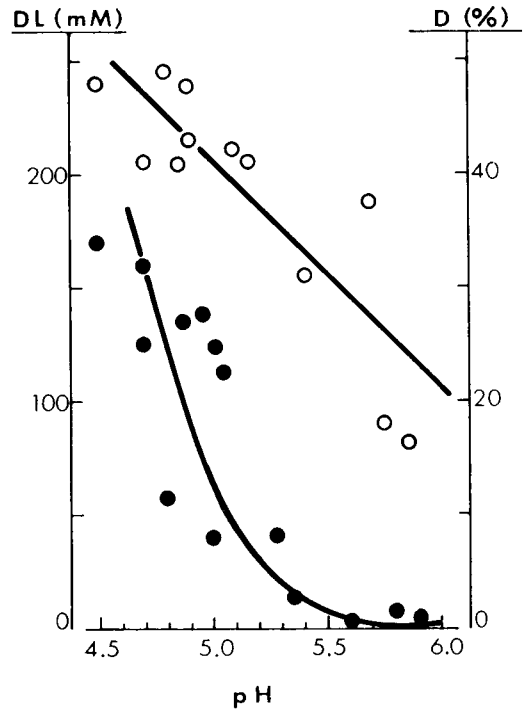

Fig 1. Rumen pH and lactate (DL—•—; D—o—) in 4 sheep overfed with maize + hay (3:1)

Kinetics of elimination

If lactic acid of similar isomer composition (45% D(-), 55% L(+) neutralized to pH 4) is injected into the blood stream, the elimination of D-lactate follows a curve composed of 4 separate exponential sections,

Table 1. Blood concentration and elimination of D-lactate in sheep and cow after intravenous injections of DL-lactate.

Blood conc. av. range, mg/l	Elimination rate constant, $k(h^{-1})$	Half-life min
10 - 60	1.386	31 ± 5 *
60 - 200	0.761	57 ± 14
200 - 450	0.348	122 ± 19
450 - 900	0.259	164 ± 27

* = Av. 10 observations

the rate constants of which correspond to 4 ranges of blood concentration between 10 and 1000 mg/l (table 1). A half-life of 90 min has been suggested for D-lactate (Huber, 1969), however, our data indicate a value of 31 min for low blood levels - which is close to that of 22 min for the L-isomer (Dunlop & Hammond, 1965) - but 4-5 fold values for high concentrations. Data for the cow and sheep were in close agreement but in goats D-lactate at similar blood levels was eliminated about twice as fast. Also the relative space of D-lactate distribution was almost identical in the former two (23,5 and 24,0% of body weight) but considerably higher in goats (31,5%). If lactic acid, after application of a priming dose, is infused continuously into the jugular vein steady state is obtained within 3-4 h. Under such conditions the infusion rate is identical with the transfer or metabolic flow through the one-compartment pool of the body. Data for sheep and goats (table 2) indicate a linear increase of the D-lactate pool and half-life with transfer up to about 5 mg/$kg^{3/4}$ min. With higher rates the elimination appeared to be drastically reduced.

Renal excretion and metabolism

It is generally assumed that D-lactate from blood is more or less exclusively eliminated via the kidneys. In sheep and goats the excretion rate (mg/kg · h=y) was linearly correlated to the blood concentration of D-lactate (mg/l=x) up to 800 mg/l by the equation

$y=0.07x-11.42 (r=0.942; p<0,001; n=11)$

corresponding to a renal threshold value of 163 mg/l. This would suggest

Table 2. Transfer and pool of D-lactate during steady state intravenous infusion of DL-lactic acid.

Sp*	Transfer mg/ $kg^{3/4}$ min	Blood conc. mg/l	Pool mg/ $kg^{3/4}$	Half-life min
S	1.43	215	100	49
G	2.45	379	243	60
G	3.54	492	347	68
G	4.72	695	524	77
S	5.47	1275	1164	147

* Sp. = species; S = sheep; G = Goat

that blood levels of D-lactate in the physiological range of L-lactate may be fully metabolized. Although tissue oxidation of D-lactate was negligible in vitro (Hinkson, Hoover & Poulton, 1967; Preston & Moller, 1973) our data of table 1 suggested that oxidation would contribute considerably to D-lactate elimination at low blood levels but less at higher ones. Infusion experiments in goats with continuous registration of $^{14}CO_2$ expiration (table 3) showed that up to about 500 mg/l D-lactate in blood the oxidation rate accounted to 30-50% of transfer.

Table 3. Relation between transfer and oxidation rate of D-lactate during steady state intravenous infusion of DL-lactic acid in goats.

Transfer mg/ $kg^{3/4}$ min	Blood conc. mg/l	Oxidation* mg/ $kg^{3/4}$ min	$\frac{\text{Oxidation}}{\text{Transfer}}$
1.98	150	0.71	0.36
2.83	379	1.27	0.45
3.54	492	1.06	0.30
4.74	695	0.92	0.19

* Calculated from expired CO_2, only

Interestingly, the maximum was found at blood levels more than twice as high as the renal threshold value. The high rate of D-lactate oxidation in vivo stimulated us to investigate the relative contribution of various organs using tissue slices in vitro (table 4). Oxidation rates were in fact low in liver but were 20fold higher in heart and kidney cortex. Relative low values for D-lactate metabolism by rumen and intestinal epithelia have been reported(Preston

& Noller, 1973).

Table 4. Oxidation of D-lactate to CO_2 by tissue slices from organs of 12 week old lambs.

Tissue slices from	No. of lambs	Oxidation /u mole C* /g DM* · h
Heart	5	130 ± 58
Kidney cortex	9	124 ± 38
Rumen epithelium	5	7.4 ± 0.6
Liver	7	5.8 ± 1.5

* C = D(-)lactic acid carbon
DM = tissue dry matter

However, oxidation is possibly not the only pathway of D-lactate metabolism. Contrary to all earlier reports our experiments with goats in vivo indicate that a remarkable proportion of D-lactate is converted into glucose. During steady state infusion of ^{14}C-(U)-D-lactate 88 and 12 uCi/mmole C were found in blood D-lactate and glucose, respectively. Whether this is a contribution of the liver or renal cortex ist still unknown.

Conclusion

D-lactate can be eliminated from the blood by renal excretion and tissue metabolism as long as urine formation is possible and the rate of D-lactate entry from the gut is not excessive. However, entry from the gut is extremely difficult to be controlled. Therefore, it is considered important to enhance the rumen utilization of D-lactate at an early stage of concentrate feeding.

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Summary of the discussion

The nature of the preferential formation of D-lactic acid at lower pH was discussed. According to the authors isomerization is pH dependant. Also the microorganisms specifically responsible for D-lactic acid formation get a better chance at low pH. The mixture used in the infusion experiments was identical to that normally present in the rumen at pH 4.5 to 5.0, and contained approx. 45% of the lactic acid in the D-form. The standard ration of hay and corn, or wheat, (1 + 3) was sufficient to provoke acidosis. Silage rations, as adapter for the microorganisms to cope with the high concentrate rations, were not tried in these studies. It may be expected that although the lactic acid content is low, the resulting lower pH, combined with any lactic acid present, will induce lactolytic bacteria.

FACTORS AFFECTING LACTATE METABOLISM IN THE RUMEN

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Summary

Of the factors which are of importance in determining the extent of lactate accumulation in the rumen during fermentation of soluble sugars the following were studied: choice of substrate, protozoal metabolism of soluble carbohydrates, rumen methanogenesis and lactate fermentation. These factors were studied in a quantitative way using pure cultures and gnotobiotic mixed cultures of rumen microbes as well as natural rumen contents.

Microbial formation of lactic acid during sugar fermentation is correlated with the rate of fermentation and the soluble sugars sucrose, fructose, glucose and raffinose support the highest rates of lactate accumulation.

Accumulation of gaseous hydrogen as a result of rapid fermentation, inhibition of methanogenesis by the lowered pH and repression of lactate fermentation by

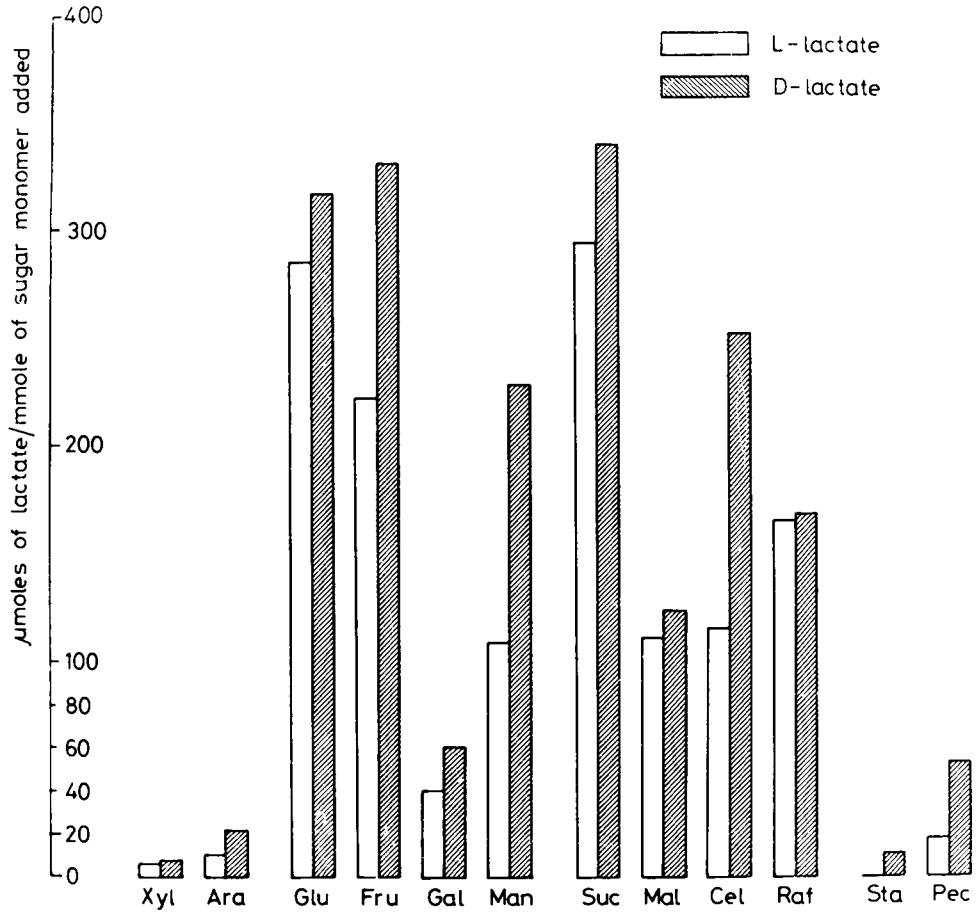

Figure 1. Accumulation of lactate isomers during fermentation of soluble sugars.

sugars and amino acids all lead to an explosive increase in the lactic acid concentration in the rumen in the early stages of lactic acidosis.

Introduction

Some species of rumen microbes may form lactic acid as one of their fermentation products, while several other species are capable of fermenting at least one of the lactate isomers to volatile fatty acids and other end-products. Nevertheless, the available evidence suggests that lactate is of minor importance as an intermediate in the rumen of roughage-fed domesticated ruminants (Hungate, 1966).

Incubation of rumen contents from ruminants (not previously exposed to diets rich in carbohydrates or lactic acid) with high concentrations of soluble sugars or readily fermentable feeds *in vitro* usually results in a rapid accumulation of lactic acid. Also, *in vivo*, whenever the rate of lactate formation exceeds the rates of removal by fermentation, absorption and passage, appreciable amounts of lactate may accumulate in the rumen. Such conditions are mostly found in over-feeding or after a sudden increase in the proportion of easily digestible carbohydrates in the ruminant diet and may lead to what is called "lactic acidosis". In this condition appreciable absorption of lactic acid from the rumen occurs, since the rumen pH is lowered. Accumulation of D-lactate in the blood is especially thought to be responsible for the acidosis which ultimately may result in the death of the animal (Dunlop, 1972).

Although the accumulation of lactic acid in itself may be viewed upon as evidence for the minor role of lactic acid as an intermediate in roughage-fed animals, we feel that the inability of the rumen organisms to cope with an increased formation of lactic acid has not been explained satisfactory. We have recently started a search for factors which are of importance in the regulation of lactate metabolism in the rumen. Factors affecting the rate of lactic acid formation as well as lactic acid fermentation are considered.

Results and discussion

Accumulation of lactic acid: influence of carbohydrate structure

When individual carbohydrates (0.2%,w/v) were incubated *in vitro* with rumen fluid from fistulated dairy cattle and when after 1 h the fluid was analyzed enzymatically for L- and D-lactic acid, it was found that appreciable amounts of both lactate isomers accumulated in the incubation fluid with some of the substrates. A typical example is shown in Fig. 1. Among the soluble sugars, sucrose, fructose, glucose and raffinose gave the highest accumulation of lactic acid, followed by maltose, cellobiose, mannose and galactose, while xylose, arabinose and also erythritol, mannitol and sorbitol (not shown in Fig. 1) gave negligible lactic acid values. The use of insoluble carbohydrates such as cellulose and xylan did not result in lactic acid accumulation, while starch and pectin gave variable results. Analysis of the rate of disappearance of the carbohydrates in the incubations showed that accumulation of lactic acid is correlated with the rate of fermentation.

Although the rate of lactic acid accumulation *in vitro* will be different with inocula from different donor animals, the relative rates with the different substrates were not much different. In other words, although the height of the bars in Fig. 1 would differ with inoculum source, the pattern of the illustration would be more or less similar for all experiments.

Formation of lactic acid: the role of the holotrich rumen protozoa

It has been a common belief, expressed many times in literature, that holotrich rumen protozoa are of value in diminishing the danger of lactic acidosis by the fact that they will rapidly form a storage polysaccharide (amylopectin) from soluble sugars and subsequently ferment this polymer slowly and primarily to volatile fatty acids. By succesfully competing with the bacteria for substrate they would prevent formation of lactic acid from at least the sugars they store as amylopectin. Experiments in our institute with purified suspensions of *Isotricha prostoma* and *Dasytricha ruminantium* showed that this is only partially true (Prins & van Hoven, 1976).

While these ciliates store a large proportion of the soluble sugar substrate as amylopectin, they simultaneously ferment free sugar to lactic acid and other fermentation products. It was found that whereas only 0.24 mole of lactate is formed per mole of endogenously fermented

amylopectin-glucose by I. prostoma, lactate becomes the major end-product of glucose fermentation when this substrate is offered as the free sugar. The results for 30 min and 60 min incubation studies are shown in Fig. 2. Rates of

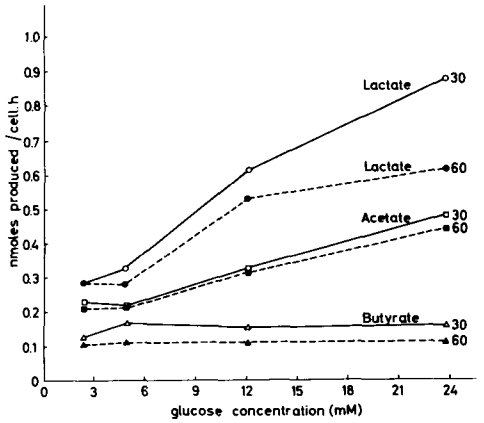

Figure 2. Rates of lactate, acetate and butyrate formation by Isotricha prostoma as a function of the initial substrate concentration.

production of acetate and lactate increase with the glucose concentration, while the production rate of butyrate remained constant in the range of the substrate concentrations tested. Similar results were obtained with other fermentable sugars. As much as 0.75 mole of lactate is formed per mole of glucose fermented at an initial glucose concentration of 2.5 mM and this figure is raised to 1.04 mole of lactate per mole of glucose at an initial glucose concentration of 24 mM in these incubations At the higher substrate concentrations D. ruminantium was almost homofermentative and lactic acid was the chief fermentation product (van Hoven & Prins, unpublished results).

The sugars most rapidly fermented by I. prostoma and D. ruminantium are fructose, glucose, sucrose and raffinose, although the latter organism also uses cellobiose, maltose and galactose at a lower rate. These sugars were also the best substrates for lactic acid accumulation with mixed rumen contents (see Fig. 1).

Formation of lactic acid: the role played by methanogenic bacteria

Earlier results (Prins & van den Vorstenbosch, 1975) showed that the formation of lactic acid as the predominant end-product from cellobiose by Eubacterium cellulosolvens, an important cellulolytic organism in the rumen of cattle, was largely prevented by co-culturing the organism with the methanogenic bacterium Methanobacterium ruminantium. Removal of gaseous hydrogen (H_2) results in a shift in the fermentation pattern of many sugar-fermenting bacteria that produce gaseous hydrogen, the chief substrate for methanogenesis. The shift is towards an enhanced production of acetate at the expense of lactate, propionate, butyrate or ethanol production.

Consequently, it could be expected that inhibition of methanogenesis in the mixed culture could result in an increased formation of reduced products including lactic acid. We were indeed able to show that this occurs using rumen fluid from hay-fed cattle. In Fig. 3 it can be seen that inhibition of methanogenesis by the potent inhibitor chloroform ($CHCl_3$) not only led to an accumulation of H_2, but also drastically increased the accumulation of lactic acid. The optimal effect

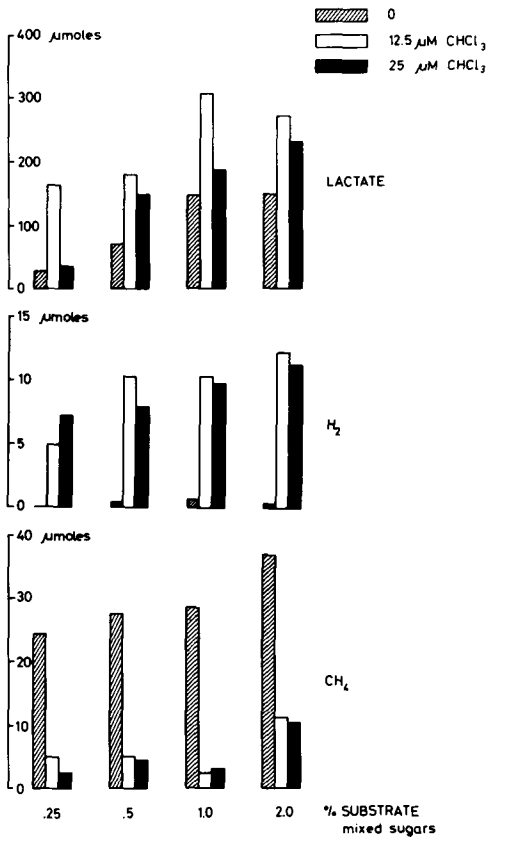

Figure 3. Accumulation of lactic acid and gaseous hydrogen as a result of inhibition of methanogenesis. An equimolar mixture of fructose, glucose, sucrose, maltose and cellobiose served as the substrate; 1 h

was achieved with 12.5 μM $CHCl_3$ while 25 μM $CHCl_3$ was probably inhibitory to fermentation in general, including the formation of lactic acid itself.

Inhibition of methanogenesis in vivo may occur during the early stages of lactic acidosis during which the rumen pH is lowered and the drop in pH would inhibit methanogenic bacteria as well as lactate-fermenting bacteria (see also Slyter, 1976 for a review). This would lead to an explosive increase in the accumulation of rumen lactate. We have also observed, however, that, even before the pH drops below pH 6, H_2 accumulates in rapid fermentations and the methanogenic bacteria can not keep pace with the highly increased rate of H_2 formation in incubations of rumen fluid from hay-fed cattle or grazing dairy cows with high concentrations of soluble sugars.

Fermentation of lactic acid: repression by sugars and amino acids

Among the predominant lactate-utilizing bacteria isolated from the rumen are: Megasphaera elsdenii, Selenomonas ruminantium var. lactilytica and Anaerovibrio lipolytica. Hishinuma et al. (1968) found that when S. ruminantium was grown in a medium containing both glucose and lactate, glucose was utilized preferentially and lactate was not fermented before the glucose was exhausted. At low glucose concentrations lactate fermentation occurred without a lag. This could mean that lactate fermentation by Selenomonas would be suppressed in vivo immediately after feeding high-carbohydrate diets.

Megasphaera elsdenii differs from the other lactate-fermenters by the fact that it produces propionate by the acrylate pathway. The other organisms use the dicarboxylic acid pathway in which succinate is an intermediate (Prins et al., 1975). Recently, we have measured the contribution of the acrylate pathway to the formation of propionate from lactate in the rumen of cattle on a grass-hay diet ad libitum with 4 kg of beet pulp daily (Prins & van der Meer, 1976). Surprisingly, the participation of the acrylate pathway to propionate formation from lactate showed a marked drop during the first 2 hours after feeding, after which there was a rapid rise to pre-feeding levels. This observation was tentatively explained as a repression of lactate fermentation by M. elsdenii by amino acids, since we had observed that this organism shows diauxic growth in a medium containing both amino acids and lactate. Repression of lactate fermentation increases the rate of lactate accumulation

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Summary of the discussion

It was added in the discussion that a ration with more sugars would favor the lactic acid accumulation, and that inhibition of the production of methane, by increased formation and accumulation of hydrogen, would add to the lactate already present.

INVESTIGATIONS ON THE PATHOGENESIS OF HYPOMAGNESAEMIC TETANY IN SHEEP

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Summary

In experiments with sheep fed different amounts of Mg the cause for the incidence of clinical symptoms in hypomagnesaemia has been investigated. In the appearance of the acute clinical symptoms neither a reduction of the Ca level in blood nor the uptake of high amounts of ammonia, phosphate or citric acid seems to be involved. On the other hand between the Mg content in CSF and clinical symptoms a strong correlation could be established. This was confirmed by perfusing the ventricular system by an artificial Mg free CSF. A small reduction of the Mg content in the intercellular fluid of the CNS may lead of functional, reversible disturbances, probably by a lower glucose uptake of the nervous cell.

During a Mg deficiency the Mg level in blood more rapidly decreases than in the CSF. The Mg in the CSF seems to buffer the brain against large fluctuations of Mg in the blood, which are typical for this mineral.

Tetanic seizures can occur, therefore, in different stages and/or after different times of hypomagnesaemia.

Introduction

Since more than 4o years it is known that severe hypomagnesaemia will often lead to tetanic signs in animals. A lowered blood Mg level seems to be an important supposition for the incidence of clinical signs, but not the real reason, because cramps will be seen at quite different blood Mg levels.

The symptoms of hypomagnesaemia have been explained by higher irritability of the end plates of the nerve-muscle-junctions. But in hypomagnesaemic animals neither a reduction of cholin esterase activity (Seekles & van Asperen, 1949) nor a higher sensitivity of the muscle by electrical stimulation (Todd & Horvarth, 197o) have been observed. On the other side the presence of some "trigger" substances for inducing acute symptoms have been postulated (Bohrmann et al., 1969; a.o.). Others (Hemingway & Ritchie, 1963) believe, that contemporary hypocalcaemia will induce the onset of tetanic signs.

To get more evidence about the pathogenesis of hypomagnesaemic tetany in ruminants the following experiments with sheep have been done, in which the influence of some "trigger" substances, the variation of the Ca blood level, and changes of the Mg metabolism of the brain in relation to the acute stages in hypomagnesaemia were investigated.

Results and discussion

Following the above mentioned observations of the connection between "trigger" substances and the onset of tetanic signs, ammonia, phosphorus and citric acid - which are known to induce clinical symptoms similar to those seen in hypomagnesaemic tetany - were infused (i.v.) in sheep with a normal or severely reduced blood Mg level. The clinical pictures during the application of citric acid and phosphate were quite different from those seen in hypomagnesaemic tetany. On the other hand the infusion of ammonia led to signs very similar to hypomagnesaemic tetany. But there was no difference in the sensitivity (time necessary to reach the maximal clinical reaction) between animals with normal and decreased blood Mg level (Meyer & Scholz, 1973; Scholz & Meyer, 1973).

Regarding the Ca level in blood in most hypomagnesaemic animals a more or less severe hypocalcaemia was observed, but no clear relationship to the onset of the acute stages (figure 1).

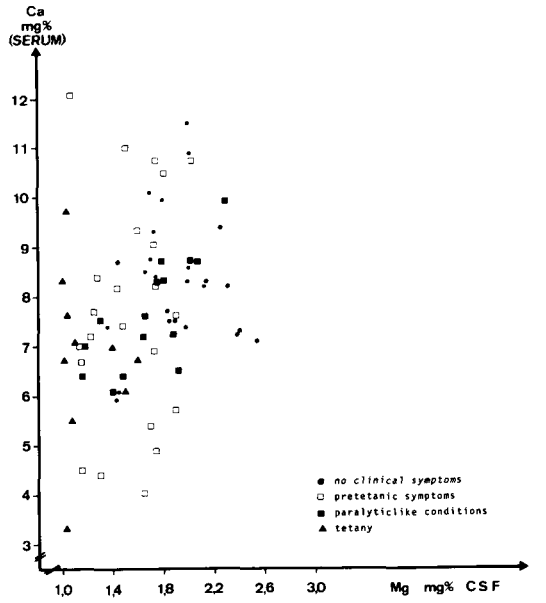

Figure 1. Relationship between blood Ca level and clinical symptoms (after Scholz & Meyer, 1972, Dtsch. tierärztl. Wschr. 79:615-619.

Some animals with severe cramps had quite normal blood Ca levels while hypomagnesaemic

ones with a severe hypocalcaemia not always reacted in a typical manner. But after our observations, contemporary hypocalcaemia may modify the clinical symptoms. In general these observations are in accordance to those of Gürtler et al. (1972) and Suttle & Field (1969).

Joining the supposition of Greenberg & Tufts (1938) and stimulated by the investigations of Chutkow & Meyers (1968) further investigations were done to see, whether the clinical symptoms during Mg deficiency were of central nervous origin and related to changes of the Mg content in the cerebrospinal fluid (CSF). The main results are given in figure 2. Tetanies only occured after the Mg in CSF declined to values lower than

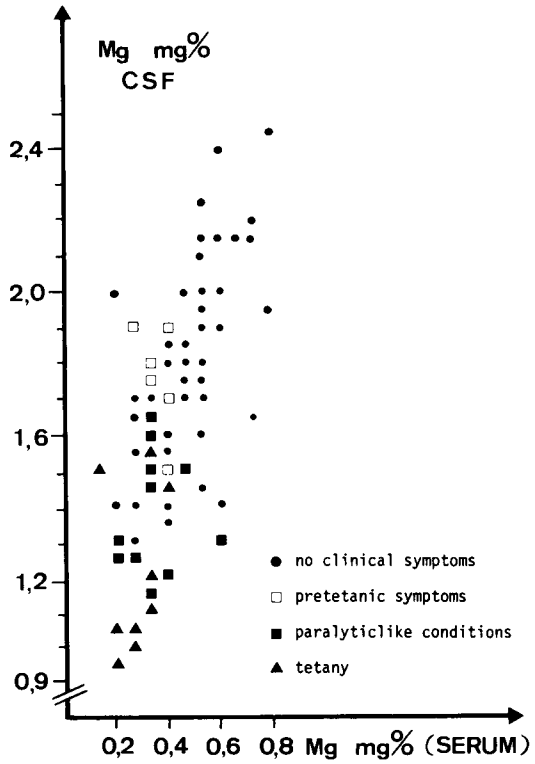

Figure 2. Relationship between the Mg level in blood and CSF and clinical symptoms (after Meyer & Scholz, 1972, Dtsch. tierärztl. Wschr. 79:55-61.)

1,6 mg % independent of the blood Mg level. Even if Mg in the blood reaches values of o,3 mg % or less, clinical symptoms were absent as long as Mg in the CSF was normal or only slightly reduced, showing that the clinical symptoms are more related to the Mg level in the CSF than in the blood.

This relationship, which has been confirmed also in field cases (Pauli & Allsop, 1974) furtheron was tested by perfusing the ventricular system of the brain with an artificial, Mg free CSF (Meyer et al., 1974). In 11 controls (blood Mg normal) no clinical reactions appeared even if the perfusion kept more than 1o hours and the Mg level in the CSF fell to values lower than o,5 mg %. In this situation the Mg requirement seems to be secured by the blood. Perfusing hypomagnesaemic sheep in the same manner however, 5 of 9 showed typical cramps which could be cured by Mg injections. During perfusion the Mg content in some compartments of the brain adjacent to the ventricular systems may have been reduced. Because 4 sheep did not react some modifying factors seems to be involved. In this connection it was a striking observation that reacting animals always have had a lower blood Mg level for longer periods before perfusion than the nonreacting ones.

For the moment we are not able to explain the further steps in the pathogenesis of hypomagnesaemic tetany. Because in hypomagnesaemic animals no (sheep, van Hattem, 1972) or only a very small (rats, Prange, 1972; Chutkow & Grabow, 1972) reduction of the whole brain Mg has been observed. There may be only a decline in the Mg content of the intercellular fluid of the brain (which amounts to about 2,5 % of the whole brain Mg) and a disturbance in the cell membrane function. By measuring the AV glucose difference between A. car. and V.jug. for example, we observed a reduced glucose uptake in hypomagnesaemic animals (Scholz & Meyer, 1976; figure 5).

Besides these questions about the pathogenesis of hypomagnesaemic symptoms on a cellular level, from a more practical point of view the relationship between blood and CSF Mg level is important. From the results given in figure 3 it can be deduced that during a Mg deficiency the Mg level in the CSF did not fall off as rapidly as the blood Mg level.

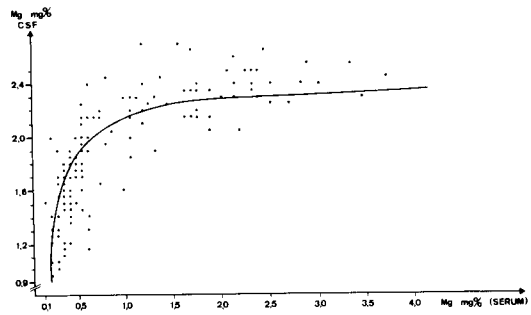

Figure 3. Relationship between the Mg content in serum and CSF in sheep (after Meyer & Scholz, 1972, l.c.)

Hypomagnesaemic animals - even severe cases - may have normal or only slightly reduced Mg values in the CSF. The Mg in the CSF seems to work like a buffer for protecting the brain against fluctuation in the blood Mg level. In general the ability of an animal to defend the Mg stock in the CSF depends on the extent

of the Mg deficiency and the possibility to mobilize Mg.

In lactating animals a more rapid reduction of the Mg in CSF can be expected than in growing lambs (figure 4). The mechanisms,

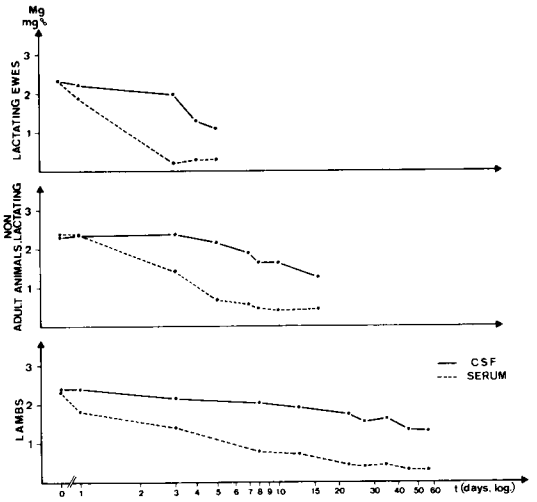

Figure 4. Reaction of the Mg content in blood and CSF during intake of low Mg diets in different classes of sheep (after Meyer & Scholz, 1972, l.c.)

which anable animals during the first time of hypomagnesaemia to hold constant the Mg in CSF for a more or less long time are till yet not clear. Because during hypomagnesaemia the Mg concentration in the freshly produced CSF is reduced (Meyer et al., 1974) the outflow of Mg from the ventricular system in the early hypomagnesaemia seems to be reduced.

In general the relationship between blood and CSF Mg level gives an explanation why animals with a low Mg blood level don't react clinically in each case or after quite different times.

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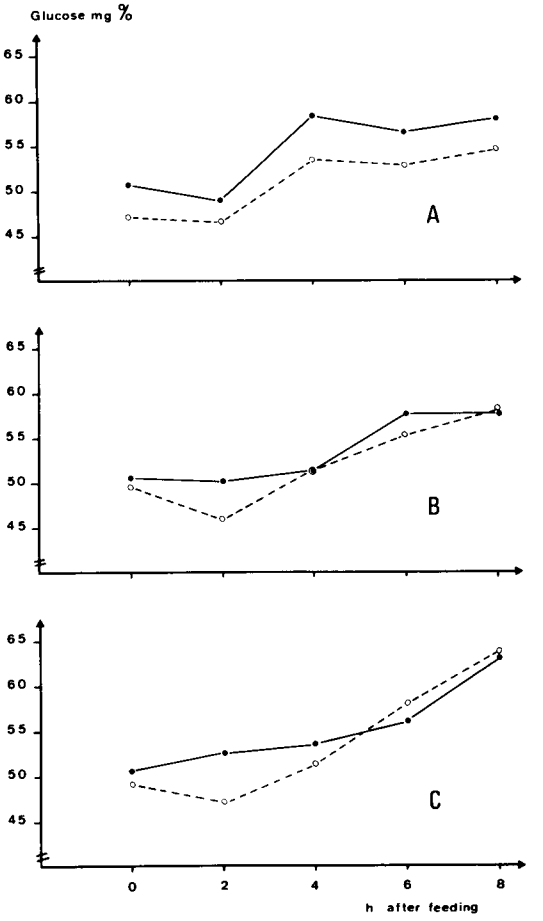

Figure 5. Glucose level in A.car. and V.jug. of sheep in different stages of hypomagnesaemia in relation to feeding time
(A = normal Mg supply, B = moderate Mg deficiency, C = severe Mg deficiency)
(after Scholz & Meyer, 1976, l.c.)

Summary of the discussion

In these experiments coupled to the decline in magnesium plasma contents no changes were found in calcium and sodium concentration. The magnesium content in the caudal part of the ventricular system was always lower than the content in the cranial part. In the nerve-muscle junctions no clearcut effects were remarked; the primary effects undoubtedly occur in the brain.

THE USE OF TABLES AND HERBAGE CHEMICAL ANALYSES TO PREDICT MEAN AVAILABILITY % OF PASTURE HERBAGE MAGNESIUM TO COW-HERDS (MINIMUM SIX COWS), AND GIVEN MEAN OBSERVED DRY MATTER INTAKE IS KNOWN, EXPECTED AVAILABLE Mg INTAKE AND AVAILABLE Mg OF RETENTION +VE OR -VE GRAMS PER DAY

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Summary

Eight Curves predicting expected blood serum magnesium concentration at varying concentrations of herbage Mg (Kemp & Rameau, 1962), redrawn partly by hand fitting around the observed mean New Zealand herbage chemical analysis for cattle-grazed swards for three elements Mg (0.226)[1] K (3.48), Crude protein (26.25), per cent of dry matter (Smith, 1968)[2], gives the twenty-three Sigmoid Prediction Curves Fig. 1. In addition, 2.3 mg/100ml serum Mg concentration is equated axiomatically with 4.9g[3] available Mg intake in 14.3 kg daily DM intake of a defined cow giving 20 kg milk daily and in zero Mg retention when grazing herbage of optimum composition. Such herbage is assumed to have a Mg concentration the abscissa of the point on any of the 23 curves Fig. 1 (or intermediate curves[4]) of value V, with ordinate 2.3 mg/100ml serum Mg concentration. These abscissae are called S_n% dm. Thus expected availability of Mg of a sward to the Notional Cow called A^p_n% is found using equation A, namely:

$$A^p_{n(v)} = \frac{4.9}{1.43 \times S_{n(v)}} \quad \% \text{ (equation A)}$$

where: V = %K x %CP, and defined mean optimum V = 52.25, giving defined mean optimum value for herbage Mg concentration

$$S_{n(52.25)} = 0.19562\% \text{ dm}$$

Thus for any observed herbage (oh), with observed Mg concentration [Mg], % dm: mathmatics show:

1. Predicted available herbage Mg intake of the Notional Cow P_n, is given by the equation.

$$P_n(P_{noh}) = 1.43 \times [Mg] \times A^p_n \quad \text{g/day}$$

2. $$P_n = 4.9 \times \frac{[Mg]}{S_n} \quad \text{g/day}$$

3. $$t_n = (A^p_n - \frac{3.42657}{[Mg]}) \times 1.43 \times [Mg] \quad \text{g/day}$$

4. $$a_n = (0.19562 - S_n) \times 25.04857 \quad \text{g/day}$$

5. $$c_n = (A^p_n - 17.51648) \times 1.43 \times [Mg] \quad \text{g/day}$$

where t_n, a_n, c_n, are predicted available herbage Mg of total, absolute and conditioned excess or deficiency to the Notional Cow and $t_n = a_n + c_n$. Finally $P_n = t_n + 4.9$g/day.

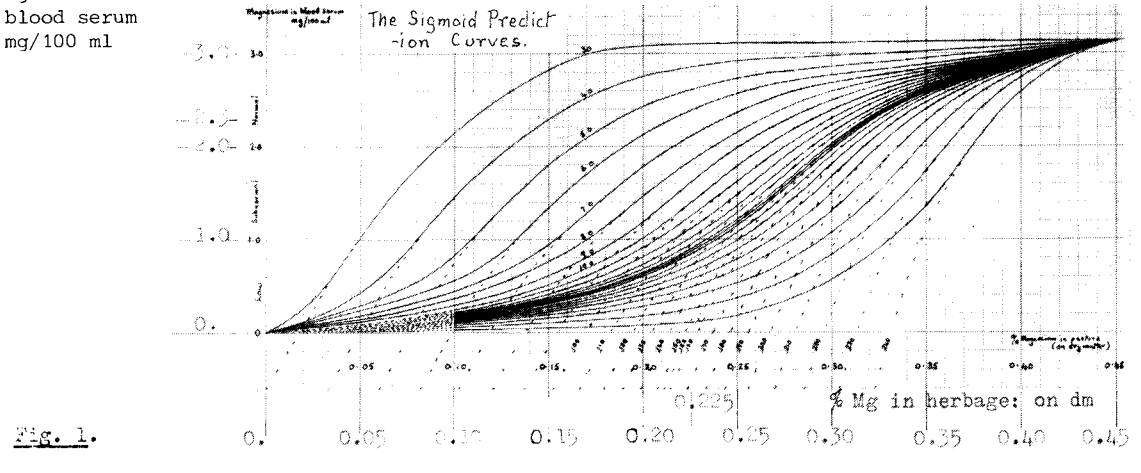

Fig. 1.

1. 0.225 was used (Fig. 1) to simplify draughtsmanship. 2. Pers comm with the Royal College of Veterinary Surgeons. 3. Calculated using the equation: available herbage Mg requirement cows in zero Mg retention (g) = 2.5 + 0.12M (Kemp & Hartmans, 1968). 4. Given a defined shift.

Introduction

Balance experiments and statistical studies employing cows and zero-grazed or grazed pas-ture herbage, are invaluable for studying the relationship between herbage chemical composition and health and production of cows, or cows and herbage. Especially when the effects of different kinds of fertilisers and rates of their application are compared. Disadvantages are high cost and duration: eight and four years respectively the Nether-lands (de Groot et al, 1973) and the U.K. (Hood, 1976). Four years can be too short to overcome the masking effects often tempo-rary, of compensatory soil, plant, and ani-mal phenomena; e.g. the ability of increase in herbage Mg concentration to offset a re-duction in Mg availability resulting from increase in herbage CP and K concentration. (Kemp et al, 1961). Since doubling herbage CP concentration is as bad an influence on serum Mg level as doubling K concentration (Kemp & Rameau 1962) a reasonably accurate prediction method giving expected availabi-lity and expected available herbage Mg con-tent of a sward, makes possible earlier eva-luation of experimental results; even the results of experiments in Europe or New Zea-land for which pasture plant data only is available, become meaningful to agronomists and veterinarians.

Results and Discussion

The Sigmoid Prediction Curves predict, for cow-groups grazing or zero-grazing unrestric-tedly unsupplemented pasture-herbage. 1. expected mean serum Mg level: 2. expected mean available Mg intake P and expected mean available Mg of retention R^P (grams).

Do predictions differ statistically non-significantly from corresponding mean obser-vations? The answer is yes to 1., but this use of the method is discussed elsewhere. Yes answers question 2., for curves with val-ues 31-74 as experiment 1. data and predic-tions, Table 1., show; using in addition equations 6 and 7 below.

6. $P = P_n \times \frac{DM}{14.3}$ g/day {where DM kg is herbage dm intake

7. $R^P = t_n + (P - P_n)$ g/day

Experiment 1. (available Mg in grams)

This is the balance experiment (Hutton & Jury, 1965: Hutton et al, 1967) using six Jersey cows zero-grazing a Perennial Rye Grass - N.Z. White Clover Sward in New Zea-land. Statistics show good agreement com-paring observations with 25 corresponding predictions, improved by grouping, correct-ing for observed digestibility, omitting week 24 values affected by previously res-tricted DM intake; especially comparing observed with predicted Mg availability.

Mean herbage analysis week 4 table 2. has the V value of Sigmoid Curve 60 Fig. 1 since %K = 3.0 and %CP = 20. Thus optimum herbage Mg level i.e. $\%S_n = 0.2155$ (tables 1. and 2.).

Table 1. $\%S_n$ values (24 of 841 tabulated) where V = Sigmoid Curve Prediction value, Fig. 1.

V: %K x %CP:	$\%S_n$ on dm	V: %K x %CP:	$\%S_n$ on dm
30.0	0.10900	140.0	0.31570
40.0	0.15050	150.0	0.31870
50.0	0.18730	155.0	0.32020
52.25	0.19562	160.0	0.32170
60.0	0.21550	170.0	0.32530
70.0	0.24250	180.0	0.32980
80.0	0.26300	190.0	0.33500
90.0	0.27700	200.0	0.34170
100.0	0.29000	210.0	0.35100
110.0	0.29970	220.0	0.36100
120.0	0.30570	230.0	0.372600
130.0	0.31120	240.0	0.385240

and using equation A, to obtain predicted Mg availability to the Notional Cow, week 4;

$$A^P_{n(60)} = \frac{4.9}{1.43 \times 0.2155} = 15.90057\%.$$

Equations 1., 2., 3., 6 and 7 give the 25 expected values P_n, t_n, P and R^P, table 2.

Now, calling predicted and observed mean Mg of urine, milk, and retention, u^P u^o, m^P m^o, and r^P r^o, we find that mean P could be distributed between requirements of the ob-served cows for maintenance, milk, and retention as follows:

$u^P(3.804) + m^P(1.096) + r^P(1.476 = 6.376$

compared with

$u^o(3.789)^* + m^o(1.082) + r^o(1.482) = 6.353$

This is because the average of the departure o of m^o from 2.4, i.e. 1.318 and of the depart-ure of u^o from 2.5 i.e., 1.289 is 1.304. This is the same value as that obtained by multi-plying r^P by 0.0106 (mean Mg concentration of milk) and dividing the product by 0.012 (defined mean Mg concentration of milk), since:

$$1.304 = 1.476 \times \frac{0.0106}{0.0120}$$

r^P and also $r^o \simeq (\text{mean } P_n \times \frac{\text{mean DM}}{14.3}) - 4.9 = 1.502$

mean P_n = 7.815: table 2.

mean DM = 11.715 kg/day; mean DM intake table 2.

*Assuming mean specific gravity of urine 1.032.

Table 2. Observations and predictions derived from the graphed data of a balance experiment (Hutton & Jury, 1965: Hutton et al, 1967) using six zero-grazed Jersey cows.

Observations in herbage and cows						Predictions derived from herbage offered					
Week No.	DM intake	Mg in herbage	herbage %K x % CP: V	available Mg intake Oc: x_2	Mg of retention R^{δ}:	availability of Mg A^{δ} % x_{2a}	availability of Mg A^{p}_{n} % x_{1a}	Required optimum herbage Mg: S_n	Notional cows available Mg intake P_n:	do, of excess def'cy t_n:	available Mg intake obsv'd cows x_1 P:	available Mg of retention R^{p}:
	Kg/day	% dm		g	g			% dm	g	g	g	g
1	11.949	0.230	61.797	2.578	-0.714	9.380	15.43067	0.22206	5.0752	+0.175	4.241	-0.659
2	11.250	.240	71.60	4.547	+0.714	16.841	13.86807	.24708	4.7595	-0.141	3.744	-1.156
3	12.286	.225	74.10	5.316	±0.0	19.230	13.56433	.25262	4.3643	-0.536	3.750	-1.150
4	12.786	.20	60.0	5.195	+0.179	20.315	15.90057	.21550	4.5476	-0.352	4.066	-0.834
5	11.357	.190	60.937	3.421	-0.357	15.864	15.63416	.21917	4.2478	-0.652	3.374	-1.526
6	11.786	.240	56.437	5.215	+1.070	18.437	16.49385	.20775	5.6607	+0.761	4.666	-0.235
7	13.286	.230	54.082	6.496	+2.857	21.258	17.03945	.20110	5.6043	+0.704	5.207	+0.307
8	13.036	.249	72.188	4.469	-0.179	13.768	13.79453	.24840	4.9118	+0.012	4.478	-0.422
9	11.250	.265	46.406	8.990	+4.820	30.155	19.36071	.17699	7.3367	+2.437	5.772	+0.872
10	10.893	.230	37.947	5.067	+0.893	20.224	23.73425	.14437	7.8062	+2.906	5.946	+1.046
11	13.036	.265	39.137	7.001	+0.982	20.266	23.11801	.14822	8.7606	+3.861	7.986	+3.086
12	13.107	.280	31.874	8.695	+2.320	23.692	28.67604	.11950	11.4819	+6.582	10.524	+5.624
13	11.071	.230	31.695	4.808	±0.0	18.882	28.87655	.11867	9.4975	+4.598	7.353	+2.453
14	11.857	.250	44.622	7.517	+2.857	25.358	20.13029	.17022	7.1966	+2.297	5.967	+1.067
15	12.321	.265	55.079	5.529	+2.143	16.934	16.83388	.20355	6.3792	+1.479	5.496	+0.596
22	13.750	.290	39.610	7.829	+1.250	19.634	22.92488	.14947	9.5069	+4.607	9.141	+4.241
24	10.179	.340	33.906	4.035	+0.357	11.659	26.61463	.12875	12.9400	+8.040	9.211	+4.311
25	10.0	.290	53.620	5.590	+0.179	19.276	17.14250	.19989	7.1090	+2.209	4.971	+0.071
26	10.714	.285	59.063	3.173	-2.054	10.391	16.02320	.21385	6.5303	+1.630	4.893	-0.007
27	10.571	.325	43.189	7.691	+2.679	22.386	20.74072	.16521	9.6392	+4.739	7.126	+2.226
28	10.714	.313	41.0	7.033	+2.143	20.972	21.97367	.15594	9.8352	+4.935	7.369	+2.469
29	11.964	.350	40.338	13.140	+7.321	31.380	22.48297	.15234	11.2527	+6.353	9.415	+4.515
30	12.857	.310	34.517	12.246	+5.714	30.725	26.17714	.13090	11.6043	+6.704	10.433	+5.533
31	10.0	.295	56.375	6.462	+1.071	21.905	16.50568	.20760	6.9629	+2.063	4.869	-0.031
32	10.857	.380	40.013	6.774	+0.804	16.419	22.75733	.15057	12.3663	+7.466	9.389	+4.489
	11.715	0.271	49.581	6.353	+1.482	19.814	19.83192	0.18199	7.8151	+2.915	6.376	+1.476

Statistics:

x_2 cf, x_1 (n = 25), r = +0.668; $f^{1.}$ = 0.003: x_{2a} cf, $x_{1\,a}$, r = +0.347; $f^{1.}$ = 0.001

" cf, " (grouped n = $13^{2.}$), r = +0.877 χ^2 = 1.854: " cf, " , r = +0.759: χ^2 = 8.060

" cf, " (" n = $13^{2.3.}$), r = +0.911 χ^2 = 1.425: " cf, " , r = +0.825: χ^2 = 3.663

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1. f is test figure of the Rapid test of Significance, the NZ Dept of Agriculture Biometrics Division, and when n = 25, non-significant difference exists when f<0.12. 2. Week 24 observations and predictions are omitted. 3. Predictions are corrected for observed digestibility of herbage.

Summary of the discussion

It was clear that too many figures were being produced to permit the audience to obtain sufficient insight needed for a reasonable discussion. It was remarked that the material is very worthwhile and interesting for further study.

CALCIUM REGULATION AND ITS RELATIONSHIPS WITH PHOSPHORUS, VITAMIN D METABOLITES, PARATHYROID HORMONE AND CALCITONIN

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Parathyroid Hormone

Although it is well established that parathyroid hormone (PTH) secretion is inversely related to both plasma calcium and magnesium concentration (Care et al., 1966; Buckle et al., 1968) more recent work has shown that the relationships are more complicated than were at first envisaged. Mayer (1975) has clearly demonstrated in calves that there is an inverse sigmoid relationship between PTH secretion rate and plasma calcium concentration. He also showed that the effect of magnesium concentration, although similar, was less effective than an equimolar concentration of calcium. At very low plasma magnesium concentrations there is not only PTH target-organ resistance but impaired PTH secretion, both presumably a result of the magnesium requirement of the adenyl cyclase involved in PTH release and subsequent action (Rude et al., 1976). This phenomenon of PTH target-organ resistance probably plays an important role in the development of the hypocalcaemia which often accompanies hypomagnesaemia and which contributes to the clinical signs of grass tetany.

Although it is now established that PTH release can be related to adenyl cyclase activity and cyclic AMP concentration in the parathyroid glands, the only hormone which can be unequivocally involved as a first messenger is adrenaline (Williams et al., 1973). The β-adrenergic component of adrenaline can also stimulate calcitonin (CT) release (Care et al., 1971) and it is possible that the sharp peak in plasma CT concentration often seen in cases of milk fever may be the result of catecholamine-induced hypersecretion of CT.

Calcitonin

The well established proportionality between CT secretion rate and plasma calcium concentration can be enhanced by several hormones e.g. gastrin (Care et al., 1975). The stimulation of CT release by these hormones (gastrin, pancreozymin-cholecystokinin and gut immunoreactive glucagon) has led to the hypothesis that CT serves not only to limit hypercalcaemia but also to protect the skeleton from excessive bone resorption during periods of dietary sufficiency of calcium (Swaminathan et al., 1973).

Vitamin D

The discovery that vitamin-D_3 must be hydroxylated twice before it can function at physiologically normal concentrations has led to a reappraisal of our understanding of calcium and phosphorus homeostasis. It is now known that vitamin-D_3, from either the diet or formed by the ultraviolet irradiation of 7-dehydrocholesterol in skin, is first hydroxylated at the 25 position by an hepatic hydroxylase to form 25-OHD_3. This is bound to a plasma α_2 globulin and transported to the kidney where a second hydroxylation at either the 1 or the 24 position is carried out. The primary biologically active form of vitamin-D_3 is 1,25-$(OH)_2D_3$. The functions of 24,25-$(OH)_2D_3$ and 25,26-$(OH)_2D_3$, both of which circulate in plasma, are at present ill-defined, as is the tissue of origin of 25,26-$(OH)_2D_3$. However, it is clear that 1,25-$(OH)_2D_3$

stimulates intestinal calcium and phosphate transport and increases the mobilization of bone calcium. Since the production of this compound is strongly feedback regulated at the physiological level it has been suggested that 1,25-$(OH)_2D_3$ should be regarded along with PTH and calcitonin (CT) as a hormone regulating calcium and phosphorus homeostasis. The activity of the renal 25-OHD_3-1-hydroxylase is repressed by 1,25-$(OH)_2D_3$ and stimulated by exposure of the renal mitochondria to lowered concentrations of calcium or phosphate ions (Bickle et al., 1975). Also the rate of biosynthesis of this enzyme is increased by raising the concentration of parathyroid hormone and decreased by parathyroidectomy (Rasmussen et al., 1972; Henry et al., 1974). In addition, Rasmussen et al. (1972) claimed that calcitonin reduces the in vitro conversion of 25-OHD_3 to 1,25-$(OH)_2D_3$ but this has not yet been confirmed by others.

Evidence for further feedback controls of calcium homeostasis has come from parathyroid perfusion studies in vivo (Bates et al., 1974; Care et al., 1975) in which the local addition of physiologically normal concentrations of 24,25-$(OH)_2D_3$ resulted in a reduction in PTH secretion rate. Since raised plasma levels of 24,25-$(OH)_2D_3$ are associated with a high calcium diet it would be appropriate for such levels to lead to a fall in PTH secretion and thereby contribute to calcium homeostasis. The position with regard to the effect of 1,25-$(OH)_2D_3$ on PTH secretion is somewhat obscure, some workers reporting inhibition (Chertow et al., 1975) and others finding stimulation of PTH release. Similar conflicting results have been obtained by us using the technique of in situ perfusion of a surgically isolated parathyroid gland in anaesthetized goats.

Adaptation of calcium and phosphate absorption from the small intestine

It is known that animals fed diets low in either calcium or phosphate increase their efficiency of calcium absorption from the small intestine (Morrissey & Wasserman, 1971) and it has been suggested that the mechanism of these adaptive effects operates through increased circulating levels of 1,25-$(OH)_2D_3$. Recent work in which rats were fed diets deficient in either calcium or phosphate showed a five-fold increase in the plasma level of 1,25-$(OH)_2D_3$ relative to controls (Haussler et al., 1976). Although thyroparathyroidectomy (TPTX) had no effect on the response to a low phosphate diet, it significantly reduced the change in plasma 1,25-$(OH)_2D_3$ level in response to a low calcium diet. This is consistent with PTH mediation of low calcium adaptation (Garabedian et al., 1972), and a direct enhancing effect of phosphate depletion on 1,25-$(OH)_2D_3$ production. Since the plasma 1,25-$(OH)_2D_3$ levels in hypocalcaemic TPTX rats were significantly higher than in relatively normocalcaemic TPTX animals (although not as high as in hypocalcaemic intact rats), it would seem that hypocalcaemia per se also increases 1,25-$(OH)_2D_3$ levels in agreement with the results of in vitro work mentioned above.

Morrissey & Wasserman (1971) found a highly positive correlation between the rate of absorption of calcium from the duodenum of chicks and the concentration of a vitamin-D-dependent calcium binding protein (CaBP) in the duodenal mucosa. Once it was established that the formation of this protein, along with enhanced uptake of calcium by the duodenum, is induced by 1,25-$(OH)_2D_3$ (Corradino, 1973), it seemed reasonable to postulate that calcium adaptation was mediated through the sequence PTH, 1,25-$(OH)_2D_3$ and CaBP. However, it has now been clearly demonstrated both in pigs (Swaminathan et al., 1974) and in rats (Favus et al., 1974) that calcium adaptation occurs in the absence of the parathyroid glands. Thus, it seems that despite the reduced level of 1,25-$(OH)_2D_3$ production in the PTX relative to the intact animal, the small rise in plasma 1,25-$(OH)_2D_3$ level in the

hypocalcaemic PTX animals is nevertheless sufficient to accomplish an increased efficiency of calcium absorption relative to its relatively normocalcaemic PTH control. The picture has been further complicated by our finding that despite adaptation of intestinal calcium absorption in PTX pigs there is no concomitant change in intestinal CaBP, as measured by a specific radioimmunoassay (Arnold et al., 1975). Moreover, although adaptation of calcium absorption in response to a diet low in calcium or phosphate might at first sight have been attributable to an increase in 1,25-$(OH)_2D_3$-mediated CaBP, this could hardly account for the adaptation of phosphate absorption which is also seen (Fox et al., 1977), since CaBP does not bind phosphate ions. In addition, this adaptation of phosphate-calcium absorption to a low phosphate diet was shown to be independent of the parathyroid glands, a conclusion one would have expected since adaptation to a low phosphate diet is associated with hypercalcaemia (when PTH secretion would be minimal). Thus, it seems likely that either a further intermediate is involved in phosphate adaptation or that 1,25-$(OH)_2D_3$ can act directly on the intestine to promote the absorption of both calcium and phosphate. The rapidity of its action on calcium absorption noted under certain circumstances (Toffolon et al., 1975; Fox & Care, 1976) supports this latter view. However, evidence in favour of an alternative mechanism for the adaptation of calcium absorption in response to a low phosphate diet has been obtained from animals maintained with either dihydrotachysterol (Bar & Wasserman, 1973) or 1,25-$(OH)_2D_3$ (Ribovich & DeLuca, 1975) as the sole source of vitamin D. Since neither sterol requires 1-hydroxylation for biological activity, it was to be expected that such animals did not show increased efficiency of calcium absorption and net synthesis of CaBP in response to a low calcium diet. However, they did show such changes in response to a low phosphate diet. It is thus clear that the mechanism of adaptation to a low calcium diet is not identical to that which occurs in response to a low phosphate intake. A somewhat analogous situation seems to exist in the kidney where it has been shown (Tröhler et al., 1976) that the dietary phosphate level seems to control a mechanism which is independent of circulating PTH and which changes the renal tubular transport capacity of phosphate ions. This effect is more potent than that of PTH but it can be amplified by PTH to allow more phosphate to be excreted during a high phosphate intake. The administration of 1,25-$(OH)_2D_3$ to a vitamin-D-deficient rat increases both the cyclic AMP content and adenyl cyclase activity in the duodenal mucosa (Walling et al., 1976). Since adenyl cyclase is calcium ion-dependent, it seems possible that the hypercalcaemia associated with a low phosphate diet might lead to enhanced production of 1,25-$(OH)_2D_3$-dependent cyclic AMP in the intestinal mucosa and a subsequent increase in permeability to both calcium and phosphate ions. It is also possible that the role of CaBP as a transport protein should be modified to that of cell protection, not only against an excessive rise in calcium ion concentration but also against inhibition of vitamin-D-dependent adenyl cyclase activity before adequate calcium and phosphate absorption has taken place.

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Summary of the discussion

The presence in the blood of the calcium binding protein in levels of approximately 1% of those existing in the mucosal cells prompts the hypothesis that it may emerge as one of the hormones of the regulation of calcium absorption in the intestinal tract. Damage to the intestinal mucosa and various other mechanisms would depress the level of the circulating "hormone". Qualitative differences between species, for instance mammals and birds, should be the subject of continuing study. Upon infusion with parathyroid hormone in doses similar to those used by Parsons in parathyroidectomized dogs, the plasma calcium level increased in a manner comparable to the measured increase in calcium absorption from the Thiry-Vella loop of jejunum.

PREVENTION OF MILK FEVER BY REGULATION OF CALCIUM AND PHOSPHORUS INTAKE AROUND PARTURITION

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Although the association between diet and milk fever has been the subject of much research for many years, only during recent years has the nature of this association become clear. The discovery of 1,25-dihydroxycholecalciferol and calcium-binding protein, and their inter-relationships with parathyroid hormone and calcitonin in calcium homeostasis, have contributed considerably to our understanding of why the dietary intake of minerals around parturition has such a dominant effect on the incidence of milk fever. Consequently, systems of feeding dairy cows have been developed to prevent milk fever which depend upon stimulating those homeostatic mechanisms which would respond to hypocalcaemia, by reducing the intake of calcium at the end of pregnancy (Westerhuis, 1974; Goings et al., 1974; Pickard, 1975).

These three feeding systems share the common feature of reduced calcium intake prior to parturition, but there are important differences. Westerhuis advocates an intake of calcium of about 30 g/day prepartum, with an increase in intake of 100 g/day after parturition and stresses the importance of feeding additional magnesium through the periparturient period. Goings et al. have shown that a very low calcium intake (about 8 g/day) from about 14 days prepartum will effectively protect cows from milk fever. These workers make no recommendations regarding calcium intake after calving.

The system which I have proposed is as follows:- from four to five weeks before calving the intake of calcium is limited to about 50 g per day and phosphorus to about 30 g per day; and beginning 2 to 3 days before calving the intake of both elements is increased by 50 g per day. This means that animals which require no supplementary feeding for 'steaming up' are maintained on roughages (hay, silage or grass) until they require additional minerals. Cows which are following a 'steaming up' programme ought to be fed concentrates which are as low as possible in calcium and phosphorus, only changing to normal dairy rations 2 or 3 days before parturition.

In experiments at the University's Farm, Jersey cows which were fed in either of these ways maintained significantly higher levels of plasma calcium at parturition than those seen in control cows which were given dairy rations in a traditional manner before calving. They also had a greater parathyroid response to hypocalcaemia than was found in control cows (Pickard et al., 1975). In farm trials involving 562 susceptible cows on 40 farms, 256 of these selected animals had previously had milk fever, but only 31 cases have occurred subsequently.

It appears that the cows adapt during the period of minimal calcium intake by increasing the efficiency of calcium absorption. The parathyroid glands are also apparently stimulated so that when the calcium level in the plasma falls at parturition, both calcium mobilisation from bone and absorption from the intestine are able to prevent the degree of hypocalcaemia from becoming severe enough to result in milk fever.

The system appears to work effectively under British conditions. An upper limit of intake of 50 g calcium per day is achieved on many farms from basic roughages since the calcium content of herbage is commonly about 0.5% D.M. and with a dry matter intake of 10 kg per day, the intake of

calcium would be about 50 g per day. There appears to be no necessity to achieve a lower intake of calcium under our conditions for adequate adaptation to take place. There also appears to be no need for additional phosphate in the diet prepartum to achieve an adequate parathyroid response. In fact a high phosphate intake could be detrimental because of its inhibitory effect on vitamin D metabolism (Tanaka & DeLuca, 1973). It is, I think, necessary to increase the intake of calcium before parturition, rather than afterwards. The requirement for calcium increases before parturition, and the timing of the increased calcium intake is estimated to coincide with the time at which levels of calcium in plasma would be starting to decline. Although this introduces management problems, and difficulty arises because of the need to estimate calving dates accurately, it is justified by the number of animals which might have milk fever before parturition and would not be covered by a change in intake occurring after calving.

There would appear to be problems associated with achieving very low calcium intakes before calving. An intake as low as 8 g per day can normally only be obtained by artificial diets. It is also theoretically possible that when the cow's reserves of calcium are low prior to introduction of a very low calcium diet she might be unable to mobilise enough bone calcium to maintain plasma calcium above the danger levels at parturition.

Problems which I have encountered so are are:-

1) Difficulty in estimating calving dates; estimation from service dates alone is too inaccurate, and in general, farmers are advised to increase the calcium intake when colostrum is obviously available in the udder. It is better to delay the time of increase until very close to calving than to feed a high calcium diet for too long.

2) Inadequate magnesium; a few cases of failure have occurred on farms where hypomagnesaemia is a traditional problem. Both PTH secretion and the responsiveness of bone are impaired by hypomagnesaemia (Rude, Oldham & Singer, 1976). Increasing the magnesium intake on such farms has been effective.

3) Excess magnesium; in their attempts to prevent hypomagnesaemia, one or two farmers were supplying too much magnesium. This was probably interfering with calcium absorption. Without knowing the intake of magnesium from the basic diet it is, I think, dangerous to supply additional magnesium to all cows before parturition.

4) High calcium levels in herbage. On a few farms the herbage may have 1% calcium in dry matter, and without supplementary feeding, this would supply too much calcium for dry cows. Increasing the calcium intake before parturition, when the intake of calcium is already too high is likely to increase the incidence of milk fever (Manston, 1967; Westerhuis, 1974). It is necessary in these cases to substitute high calcium herbage with low calcium cereals if milk fever is to be prevented by dietary means.

The incidence of milk fever is, in our experience, likely to be low when the intakes of calcium, phosphorus and magnesium are close to the cow's requirements for these elements around parturition. The feeding system I have proposed is based on this concept, and on the fact that under practical farming conditions it is difficult to achieve low intakes of calcium during the dry period. It is my view that most cows can cope with slight deviations from requirements and the most important point to note in the prevention of milk fever is the need to avoid such excesses of calcium intake as commonly occur when large amounts of dairy rations or high-calcium herbage are fed to dry cows.

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Summary of the discussion

Some practical examples were given of the recommended diets supplying 50 g or less of calcium per day. The relative excess of calcium in roughage may be balanced by the shortage in the concentrates. The recommended ration generally is in line with that for the prevention of ketosis. A test for prediction of milk fever is not yet available; the analysis of 1:25 OH cholecalciferol is too complicated for general use.

ADAPTATION OF CALCIUM ABSORPTION FROM THE GUT OF COWS AT THE ONSET OF LACTATION

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Introduction

The period in which calcium homeostasis of cows is most severely challenged and most subject to failure occurs within a day or two of parturition. At that time cows fed the usual rations become highly dependent on absorption of calcium from the gut to maintain calcium homeostasis especially as they get older. Studies of the kinetics of calcium before and after parturition of sheep (Braithwaite et al., 1969) and cows (Ramberg et al., 1970) by combined nutrition balance and ^{45}Ca radioactivity measurements have shown that the absorption of calcium from the gut increased early after the onset of lactation. This adaptation of calcium absorption is apparently not sufficient to compensate for the massive outflow of calcium into the mammary gland during the first part of the lactation period as it is generally found that cows after parturition are in negative calcium balance, regardless of the amount of calcium supplied in the diets (Ellenberger et al., 1931; Ward et al., 1952; Duncan, 1958). More detailed information on the degree and speed of adaptation of calcium absorption post partum may be helpful in finding methods to prevent milkfever.

Conventional balance studies over periods as short as 2 days can not accurately evaluate the changes in absorption that occur in that time. Also in studies in which a radionuclide of calcium was given orally a delay of many hours in the absorption of the tracer was found in adult cows (Ramberg et al. 1972). It probably reflects delay and dilution in the rumen and the need of transport to the active site of absorption in the small intestine.

In the experiments to be presented here the absorption of calcium was measured by infusion of ^{45}Ca into the duodenum of cows during 9 to 12 h periods. The unabsorbed ^{45}Ca reaching the end of the small intestine was measured. For more details see Van 't Klooster (1976). The ^{45}Ca infusions and samplings of ileal digesta were done repeatedly pre- and postpartum in cows with constant intakes of calcium during the whole period.

Methods

The experiments were made with 5 dairy cows, aged 4 to 9 years, fitted with T-piece cannulae in the distal duodenum and with re-entrant cannulae in the ileum at less than 50 cm distance from the caecum. The rations, composed of hay (8 kg per day) and concentrates were offered in equal portions at 6 a.m. and 6 p.m. The Ca-intake was kept constant during the experimental periods lasting from 1 month pre-partum till 1 month post-partum by supplying the cows with $CaHPO_4$ twice a day via the rumen cannulae. For 3 cows the Ca intake was approximately 72 g per day. One cow (W) received approximately 80 g Ca per day and one cow (A) 62 g per day during the whole experimental period. Because of variations in refusals, especially around parturition (0.5-3 kg per day, mostly hay) the Ca intakes varied with the amounts of Ca contained in the refusals.

The absorption of ^{45}Ca from the small intestine was estimated on a varying number of days during the period of two weeks pre- and 4 weeks post-partum. A solution containing 50 g PEG and 50 μ Ci ^{45}Ca per l was pumped into the duodenum via a T-piece cannula from 6.00 h a.m. till about 17.00 h p.m. Ileal digesta was collected from 9.00 till about 18.00 h , weighed and sampled proportionally (5%). From the ratios of ^{45}Ca: PEG in the infusion solutions and in the ileal digesta samples, the percentage absorption of ^{45}Ca was calculated (Van 't Klooster, 1976).

Results

As shown in fig. 1. about 20% of the ^{45}Ca infused into the duodenum was absorbed from the small intestine in 4 out of 5 experimental animals pre-partum. At this stage the cow (E) with the lower calcium intake (62 g/day) absorbed over 30% of the ^{45}Ca.

After parturition the absorption percentages increased in all cows but the increase was faster and showed a shorter lag time in the experiments with cows A, D and E than in the experiments with cows B and C. The latter two cows were slow to eat after parturition and showed vague symptoms of hypocalcaemia. They were injected with calcium borogluconate after finishing the experiment at the day of parturition and recovered. The plasma calcium levels of the cows treated for hypocalcaemia differed only slightly with those of the untreated cows.

The strongest increase in absorption percentages was seen in the first week of lactation as did the increase in milk production of the experimental animals.

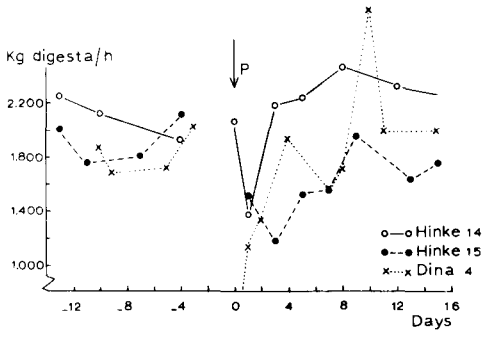

Fig. 1. The quantities of digesta (kg/h) that passed through the ileal re-entrant cannulae of 3 experimental cows during the experimental period of 2 weeks prepartum and 4 weeks postpartum. Note the variable passage rate of around parturition.

After the first week of lactation the absorption percentages fluctuated considerably but over the whole they increased only slightly after day 8 postpartum.

On the day of calving the flow rate of digesta through the ileal cannulae was low compared with other days pre- and postpartum except in cow A (fig. 2).

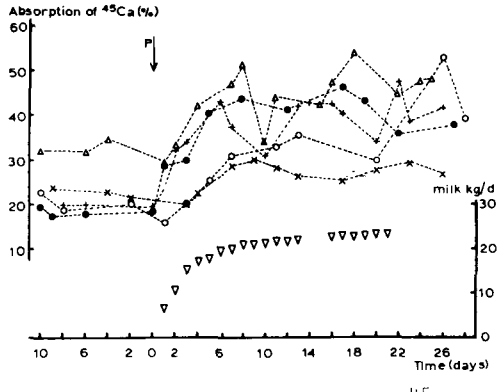

Fig. 2. Absorption percentages of ^{45}Ca prepartum and postpartum of 5 cows and the mean milkproduction of these cows. ●---● cow A, o---o cow B, x---x cow C, +---+ cow D and Δ---Δ cow E.

In the experiments with cow B and C hardly any digesta passed the ileal cannulae on the day of calving so that absorption of ^{45}Ca could not be measured with the method used. The day after these cows were treated with calcium borogluconate, the passage rate of digesta was still low but low values were also found in the experiments with the other cows.

A plot of the percentage absorption of ^{45}Ca against calcium secretion in the milk in g per day (fig. 3) indicated a linear relationship with a regression coefficient between animals of 1.12 standard deviation 0.137 ($P < 0.001$).

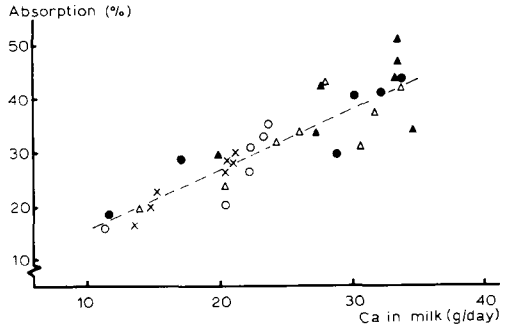

Fig. 3. Relationship between absorption efficiency (A) of ^{45}Ca and secretion of calcium in the milk (M). $A = 4.89 + 1.12\ M$, $P < 0.01$.

Discussion

The results obtained in the present experiments compare favourably with the results presented by Ramberg et al. (1970). These authors also found an increase in calcium absorption from the gut of cows in the first week postpartum but they concluded from their results that a decrease in absorption in the second week postpartum coincided with an increase in calcium removal from the bone. Such a decrease in absorption was not found in the present experiments, although two of the cows (D and E) showed a transient decrease in absorption 10 days postpartum.

The calcium absorption in our experiments increased in 3 of the cows with a delay of about 1 day. This fits in with the suggestion of Ramberg et al. (1972) based on model simulations that there is a delay of about 24 h before the inflow increases after the onset of lactation. This delay was longer in the experiments with the 2 cows injected with calcium borogluconate. As only 7.4 g of calcium were injected it is not likely that the injection is responsible for the slower adaptation. As the milk production of these 2 cows was lower than the average production of the 3 other animals and as calcium secretion with the milk and the efficiency of absorption were highly related, the differences in adaptation pattern may reflect the differences in milk production.

If it is assumed that the absorptive efficiency for the tracer and stable calcium is identical, a 10% increase in absorption efficiency can be expected for each 8.9 g calcium secreted in the milk. With a calcium intake of 72 g per day a 10% increase in

absorption efficiency would result in an increased inflow of 7.2 g calcium into the blood, which would cover about 80% of the calcium secreted. However, most of the endogenous calcium is secreted proximal to the absorption sites and it seems likely that the calcium contained in saliva, gastric juice, bile and pancreatic juice is absorbed as efficiently as the calcium fed. A more efficient utilization of the endogenous calcium will decrease the difference between the inflow of calcium from the gut and the secretion with the milk, but will not abolish it. Some calcium -about 10 to 15% of the calcium secreted with the milk- must have been removed from the bone. It is suggested (Ramberg, 1972) that in cows fed low calcium diets prepartum, calcium removal from bone can be of much more importance. A high level of calcium removal from bone will be of great value to the animal at the onset of lactation, especially when the absorption is depressed by hypophagia and hypomotility of the gut (Moodie and Robertson, 1961) or by other factors.

Feeding low calcium diets prepartum not only improves the rate of release of calcium from bone but also conditions the cow to absorb the calcium fed more efficiently. In 28 balance experiments with 4 lactating cows Van Leeuwen and De Visser (1976) found mean apparent absorption coefficients for calcium of 70% (range 63 to 73%) when rations were fed with calcium contents as low 0.22% (range 0.17 to 0.25%). When rations with 0.44% calcium in the dry matter were fed to the same cows the apparent absorption coefficients were on average 33.6%. There seems to exist an inverse relationship between absorption efficiency and calcium intake.

When the calcium intake is increased immediately after parturition by feeding a high calcium ration from parturition on or by dosing calcium orally (Van Meurs, 1974; Westerhuis, 1973) the inflow of calcium from the gut may be increased at the onset of lactation. The effect of a high orally dose of calcium on the adaptation of calcium absorption remains to be investigated.

Although it is likely that by feeding low calcium diets prepartum the frequency of cases of milkfever can be reduced (Westerhuis, 1973; Wiggers et al., 1974) this may present practical difficulties because of the relatively high calcium content of the common feedstuffs and the low requirements of dry cows.

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Summary of the discussion

Independant evidence (Care) exists for the persistance of the increased calcium absorption for a few days after withdrawal of the stimulus. The author added that also in other experiments, with EDTA infusion, persistant higher absorption efficiencies were obtained. Such persistance is very important for the practical measures to prevent milk fever. If shortly before calving the low calcium/phosphate diet is replaced by a diet with higher calcium/phosphate ratio, the persisting high efficiency of calcium absorption will result in an enormously increased total calcium accretion during the 2-3 day's critical period, at calving and immediately thereafter. For stimulation, at least theoretically, also calcium complexing compounds could be fed in the dry period, but oxalic acid is excluded because it will not escape digestion in the rumen. In the dairy cows used in the experiment plasma calcium after parturation was lower than before, so that only at that time the adaptation mechanism was started. No relation could be found with the efficiency of absorption in this experiment, but in other trials it could be shown to exist on varying calcium intakes. Replacing a high for a low calcium diet induced a decrease of plasma calcium levels, which resulted in higher efficiency of calcium absorption.

THE POTENTIAL VALUE OF 1α-OH CHOLECALCIFEROL FOR THE PREVENTION OR TREATMENT OF MILK FEVER

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Summary

When cows were treated with 250 μg 1α-OH cholecalciferol (1α-OH D_3) within 2 hours after having their third or subsequent calf, they showed significantly less post-parturient hypocalcaemia and hypophosphataemia than untreated cows.

When the same dose of 1α-OH D_3 was administered as nearly as possible 24 hours before calving the post-parturient hypocalcaemia and hypophosphataemia were almost completely prevented.

1α-OH D_3 may be valuable for the prevention or treatment of milk fever.

Introduction

Vitamin D_3 has long been used for the prevention of milk fever (Seekles et al., 1958) because it increases both the absorption of calcium and phosphorus from the diet and the mobilisation of these elements from bone. However, these effects are induced slowly and the vitamin must therefore be administered at least 3 days before calving - a difficult period to judge accurately. 1α-OH cholecalciferol (1α-OH D_3) is an easily synthesised analogue of the physiologically active metabolites of vitamin D_3 (Holick et al., 1973) which stimulates the mobilisation of calcium and phosphorus from diet and bone more quickly than vitamin D_3 itself, and thus may be more useful for the prevention or treatment of milk fever. Recently doses of 1.0 μg 1α-OH D_3/kg bodyweight have been shown to increase plasma calcium and phosphorus concentrations in pregnant dairy heifers (Sansom et al., 1976) and doses ranging from 1.72 to 14.7 μg/kg have produced similar effects in calves, steers and lactating cows (Barlet, 1975).

We have conducted two field trials in order to test the effectiveness of 1α-OH D_3 in preventing the post-parturient hypocalcaemia and hypophosphataemia which occur normally in cows.

Materials and methods

In both trials Friesian cows from within the I.R.A.D.'s herds, having their third or subsequent calves were used. The first trial took place between December 1975 and March 1976 when the dry cows' diet contained approximately 63 g calcium, 30 g phosphorus and 62 g magnesium per day and was supplemented with vitamin D_3 according to A.R.C. recommendations (1965). The second trial took place between April and August 1976 and during this period the dry cows were grazing and received no supplementary feed.

Trial I

Twenty cows were used. Blood samples were taken from a jugular vein into heparin, on days 5, 2 and 1 before the expected date of calving and then daily if this were delayed. Within 2 hours after calving another blood sample was taken and the cows were given 250 μg 1α-OH D_3 in 2.5 ml carrier or 2.5 ml of carrier alone, injected into the neck. Treated and control cows were selected from pairs of cows in order of calving. Blood samples were then taken between 9-18, 19-35 hours and on days 2, 3, 4, 5, 10 and 15 after calving.

Trial II

Twenty cows were used. Blood samples were taken from a jugular vein daily from the 5th day before the predicted calving date. On the day before the cow was judged to be going to calve 250 μg 1α-OH D_3 in 2.5 ml carrier or 2.5 ml of carrier alone was injected subcutaneously and blood samples were taken daily until 5 days after calving, and also 10 and 15 days after calving. If a cow calved early 1α-OH D_3 or placebo was administered not later than 6 hours after calving and if a cow did not calve within 72 hours of receiving 1α-OH D_3 it was given a second dose. Treated and control cows (T and C respectively) were at first selected in the order TCTTCTT but subsequently treated and control cows were selected alternately. 11 cows received 1α-OH D_3 and 9 cows received the placebo.

In both trials plasma was separated from the blood samples and the concentrations of calcium, magnesium and phosphorus were determined - calcium and magnesium by atomic absorption spectrophotometry and phosphorus by an automated method (Manston, 1966).

Results

Trial I

The mean plasma concentrations of calcium and phosphorus in 9 of the treated and the 10 control cows during the 5 days before calving and the 5 days after calving are shown in Figs. 1 and 2 respectively.

Figure 1. The mean plasma concentrations of calcium in 9 cows treated with 250 µg 1α-OH D_3 within 2 hours after calving and 10 control cows.

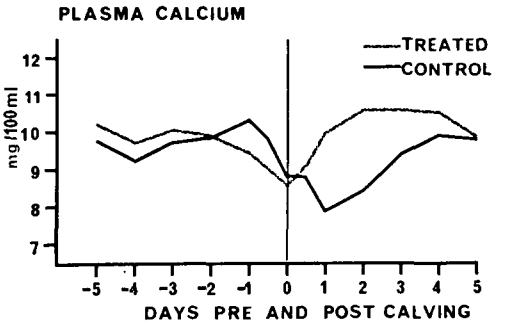
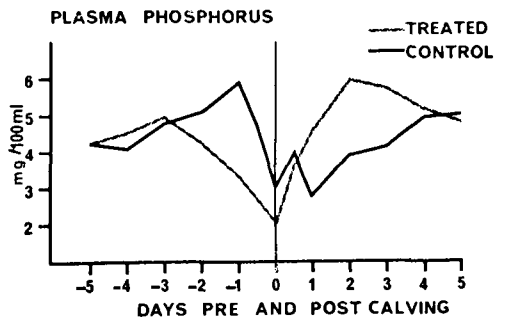


Figure 2. The mean plasma concentrations of phosphorus in 9 cows treated with 250 µg 1α-OH D_3 within 2 hours after calving and 10 control cows.



A moderate hypocalcaemia and hypophosphataemia occurred in all cows at calving, but in the control cows the minimum concentrations of calcium and phosphorus were not reached until 24 hours after calving whereas by this time the cows treated with 1α-OH D_3 had normal plasma calcium and phosphorus concentrations. In the untreated cows plasma concentrations of both calcium and phosphorus took 3-4 days to return to normal. Between 18 and 84 hours after administration of 1α-OH D_3 there were significant increases in the plasma calcium ($p<0.001$) and phosphorus ($p<0.01$) concentrations of the treated cows. During the same period the cows receiving 1α-OH D_3 tended to have decreased plasma magnesium concentrations.

One cow which received 1α-OH D_3 suffered clinical milk fever. Her plasma calcium concentration at calving was 5 mg/100 ml and the administration of 1α-OH D_3 at calving did not prevent a further slight decline which necessitated treatment 12 hours later with calcium borogluconate.

Trial II

Two of the treated group of cows received 2 doses of 1α-OH D_3 and two of the control cows suffered clinical milk fever. All results from the former 2 cows and results from the latter 2 cows after they were treated with calcium borogluconate are excluded from Figs. 3 and 4 which show the mean plasma concentrations of calcium and phosphorus respectively in the remaining experimental animals from 5 days before to 5 days after calving.

Figure 3. The mean plasma concentrations of calcium in 9 cows treated with 250 µg 1α-OH D_3 approximately 24 hours before calving and 9 control cows (results from 2 control cows omitted after calving).

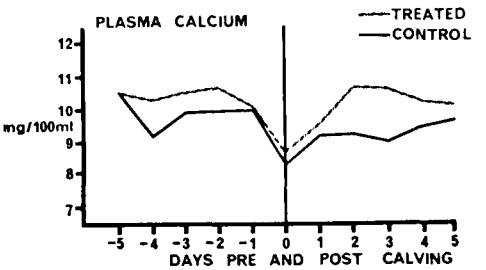
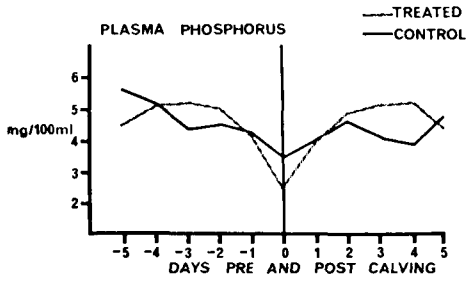
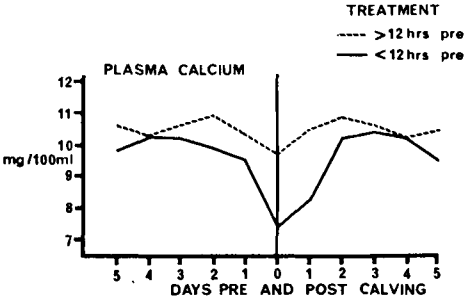


Figure 4. The mean plasma concentrations of phosphorus in 9 cows treated with 250μg 1α-OH D_3 approximately 24 hours before calving and 9 control cows (results from 2 control cows omitted after calving).



The results were similar to those obtained in the first trial. Both control and treated cows had a moderate hypocalcaemia and hypophosphataemia at calving, from which the treated cows recovered more rapidly. However, the results at calving are biased by the results from 4 cows which were treated only shortly (mean 6 hours) before calving. In Fig. 5 the mean plasma concentrations of these cows are compared with those of the 5 cows which were treated with 1α-OH D_3 an average of 40 hours before calving.

Figure 5. The mean plasma concentrations of calcium in 5 cows treated with 250 μg 1α-OH D_3 more than 12 hours before calving and in 4 cows treated less than 12 hours before calving.



In the latter group the hypocalcaemia at calving was almost completely prevented, the lowest plasma calcium concentration being 9.2 mg/100 ml.

In the second trial there was no difference in plasma magnesium concentration between the treated and control cows.

Discussion

In Trial I cows treated with 250 μg 1α-OH D_3 administered within 2 hours after calving suffered significantly less post parturient hypocalcaemia and hypophosphataemia than control cows in which these conditions persisted for up to 72 hours after calving. However, approximately 45% of milk fever cases occur before or within 24 hours after calving (Mullen, 1975) and most of these could not be prevented by the administration of 1α-OH D_3 at calving. This problem is emphasised by the occurrence of a case of milk fever among the cows receiving 1α-OH D_3.

One way of trying to overcome the difficulty would be to administer 1α-OH D_3 approximately 24 hours before calving, and this method was adopted in Trial II. The 5 cows which were treated between 23 and 66 hours before calving suffered practically no hypocalcaemia at or after calving, but the 4 cows treated less than 12 hours before calving still suffered significant hypocalcaemia and hypophosphataemia. However, there were no cases of milk fever among the treated cows, whereas there were 2 cases among the nine control cows.

Larger doses of 1α-OH D_3 raise plasma calcium and phosphorus concentrations for longer periods (Sansom et al., 1976). Such doses would therefore tend to make the time of administration before calving less critical in determining the efficacy of 1α-OH D_3 in preventing milk fever. A field trial has recently begun, using approximately 1,000 cows to each of which a dose of 500 μg 1α-OH D_3 will be administered as nearly as possible 24 hours before calving. The results of the two small trials reported here suggest that this treatment should give a useful protective action against milk fever.

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Summary of the discussion

Although in the second field trial the treated group dosed more than 12 h pre-partum had similar increases in serum calcium as the group dosed less than 12 h pre-partum, the effects at the calving date were better for the first group. This looks promising for further attempts at milk fever prevention. The cost of treatment with 1α-OH D_3, though uncertain at the moment, are probably much less than those for the systems involving feeding low calcium diets to dry cows. The quantity needed, according to work in Compton, are rather small (1 µg/kg body weight). Feeding low calcium diets pre-partum especially is difficult in grass feeding. In Sweden (Jönsson) much more was needed, approx. 4 times as much as found in Compton. Such doses not only are expensive, but they also can cause serious side effects resembling those of hypervitaminosis D_3. Pathological eye symptoms were even observed on the lower dose of 1 µg/kg. These symptoms probably are not caused by particular inbreeding, but genetic differences are certainly not excluded. It was suggested (Payne) that veterinary surgeons diagnose the operative aetiological factors for each herd outbreak of milk fever. Then attempt to correct this factor, either by dietary adjustment or by specific prophylaxis. It is most unlikely that any one method of prevention will be effective for all farms.

AN ATTEMPT TO PREVENT MILK FEVER

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Summary

In three consecutive years the incidence of clinical milk fever in a Friesian dairy herd was 29, 32 & 22 percent of third or later calvings. To assess the influence of diet, half of the grazing pregnant cows were each fed, during the last month of pregnancy, a daily supplement of 5 lbs rolled barley and the other half each received a daily supplement of 5 lbs of a concentrate mixture. A few days before parturition all cows were given 8 lbs of dairy cake per day. During a period of 4 months 29% of the barley supplemented cows and 18% of the concentrate supplemented cows developed milk fever.

Introduction

There are numerous reports of the effect of the intake of calcium and phosphorus and of the ratio of calcium to phosphorus in the diet on the incidence of milk fever (hypocalcaemia) in dairy cattle. Many references to work in this field are given by Jorgensen (1974). Kendall, et al., (1966) reported a reduction in the incidence of milk fever when grain was fed to the extent of 1% of the animal's bodyweight during the period before parturition. More recently Pickard (1975) advocated an increase in the intake of calcium and phosphorus by the addition of acid calcium phosphate to the diet from two to three days before the estimated date of calving.

We have attempted to combine the effects of both these dietary modifications in a trial carried out on a Friesian herd in which the incidence of clinical milk fever in three consecutive years was 29, 32 & 22 percent of third or later calvings.

Methods

The trial was carried out during the four months of August to November inclusive of 1975. During this period approximately half of the cows which had previously produced at least two calves were fed a supplement of five pounds of barley per day (Group A) and the other half (Group B) received five pounds per day of a concentrate mixture during the first three weeks of the last month of pregnancy. When calving was estimated to be due within seven days, the cows of both groups were given 8 lbs of dairy cake per day. Both groups shared the same pasture throughout the trial and the mineral content of the grass was analysed each week.

Assuming an average daily intake of grass and concentrate equivalent to 13 kg of dry matter (Corbett, 1969) during the three week period, the average daily intakes of calcium and phosphorus by Group A cows were 71g and 53g, giving a Ca/P ratio of 1.3 and the average daily intakes of calcium and phosphorus of Group B cows were 106g and 59g, giving a Ca/P ratio of 1.8. Assuming that the availabilities of calcium and phosphorus in grass are 45% and 55% respectively (Agricultural Research Council, 1965), in barley 30% and in the concentrate 61% (T. L. J. Lawrence, personal communication), the daily intakes of available calcium and phosphorus by Group A cows were 32g and 27g, giving a Ca/P ratio of 1.2, and for Group B cows 48g and 34g, giving a Ca/P ratio of 1.4. These intakes are in excess of those recommended by the Agricultural Research Council (1965), i.e. calcium net 33g (available 15g) and phosphorus net 34g (available 18.4g).

During the last week of pregnancy, when both groups of cows were given a daily supplement of 8 lbs of dairy cake, in addition to their grazing, the average daily intakes of calcium and phosphorus became 99g and 62g respectively, giving a ratio of 1.6. The daily available calcium and phosphorus intakes were 46g and 36g with a ratio of 1.4. Therefore, the ratio of calcium to phosphorus in the diet of Group A cows changed from 1.3 to 1.6 and in the diet of Group B from 1.8 to 1.6 at one week before calving. The calcium and phosphorus contents of the various diets are summarised in Table 1.

TABLE 1 AVERAGE INTAKES (g/day)

	Actual			Available		
	Ca	P	Ca/P	Ca	P	Ca/P
Group A (Barley 3 weeks)	71	53	1.3	32	27	1.2
Group B (Concentrate 3 weeks)	106	59	1.8	48	34	1.4
Both Groups (1 week)	99	62	1.6	46	36	1.3
A.R.C. Recommendations	33	34	1.0	15	18.4	0.8

Results

The feeding trial continued for four months. Every diagnosis of milk fever was confirmed by serum analysis. There were 14 cases of milk fever out of 49 calvings (29%) in Group A (barley supplemented) and 9 cases out of 51 calvings (18%) in Group B (concentrate supplemented). This difference is not significant.

Discussion

When considering earlier reports on the effect of dietary changes on the incidence of milk fever, it is difficult to assess the part played by changes in the absolute intakes of calcium or phosphorus and by changes in the ratio of dietary calcium to phosphorus. Preliminary calculations made during the planning of the present trial suggested that the feeding of barley would reduce the calcium and phosphorus content of the diet of Group A animals to a greater extent than was finally achieved. In the event, the mineral content of the grass was such that the intakes were those given in Table I. Table II compares the daily dietary intake of Group A animals with Pickard's latest recommendations (1976).

TABLE II — AVERAGE INTAKES (g/day)

	Group A		Pickard	
	Ca	P	Ca	P
3 - 4 weeks before calving	71	53	50	30
7 days before calving	99	62	100	80

The table shows that although it was not possible to depress calcium intake three weeks before calving to Pickard's level, our animals received his suggested intake of calcium in the immediate pre-parturition period. Phosphorus intake was less than his recommendation.

The results of this experiment suggest that in those regions of the world where autumn calving of grazing cows is practised, control of milk fever by dietary regulation will continue to be difficult.

Another problem arises when an attempt is made to change the diet of cows a few days before the day on which calving takes place. However good the farm records are, individual calving dates will vary and there will be a wide range of intervals between the change of diet and the onset of parturition in the individual animals in any herd.

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Summary of the discussion

The formulation of a low calcium diet, especially if the main component is grass, for dry cows is fairly difficult. Even quantities as low as 50 g calcium per cow per day may not be sufficiently low for stimulation of 1.25 dihydrochelecalciferol, but it is about the limit of practically available low calcium diets. In the calculations of this paper the available calcium was for each type of food obtained by multiplication of the actual calcium intake by the known availability percentage.

PARATHYROID HORMONE RELEASE IN CATTLE REGULATED BY CALCIUM AND CATECHOLAMINES AND RESPONSES DURING POSTPARTURIENT HYPOCALCEMIA

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Summary

Hypocalcemia and catecholamines directly stimulate and have separate effects on parathyroid hormone release. However,interactions between the two stimulators can be demonstrated and may be important in postparturient hypocalcemia.

Introduction

Serum Ca and Mg concentrations are the most important factors regulating parathyroid hormone(PTH) secretion. Catecholamines stimulate PTH release in cows(Fischer et al.,1973 a).

This communication summarizes our experiments designed to further characterize PTH responses regulated or modulated by Ca and catecholamines and relates the findings to investigations on parathyroid function during postparturient hypocalcemia.

Results and discussion

1.Stimulation

During abrupt hypocalcemias(EGTA infusions),PTH responses were biphasic with an initial peak occuring at 4-8 Min(acute or early response), as previously described(Fischer et al.,1973 b;Blum et al.,1974 a).PTH subsequently decreased,then gradually increased again(late response).As during postparturient hypocalcemia (Blum et al.,1974 b),the early responses were not linearly related to serum Ca.Basal PTH concentrations and the magnitude of the early and late responses during EGTA infusions could be reduced,if lactating cows received high calcium supplements in their diets.However,high calcium supplements given to pregnant cows did not seem to markedly affect parathyroid function during postparturient hypocalcemia.

Epinephrine-(and isoproterenol) infusions stimulated mainly the early PTH response and despite of continuous stimulation,PTH decreased towards basal concentrations and additional epinephrine infusions induced no additional PTH release.At this refractory state EGTA infusions however provoked a spiking release.Resensitation occured within less than two hours.Infusions of α-adrenergic blockers stimulated PTH release within Minutes.

2.Inhibition

During calcium infusions PTH concentrations decreased within Min,but PTH levels were not completely suppressed.

β-adrenergic blockers depressed PTH concentrations due to an increase of total and ionized Ca.

3.Interactions

Epinephrine could not stimulate PTH release in hypercalcemic cows.On the other hand,when parathyroid glands were maximally stimulated with EGTA infusions and epinephrine infusions were then superimposed, acute responses were up to 3 times higher than during normocalcemia.

When epinephrine and isoproterenol, but not when norepinephrine(in equal molar amounts as epinephrine) were infused and EGTA infusions were then superimposed,a potentiating effect was also observed.

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Summary of the discussion

In order to keep the calcium intake as low as 10 g per cow per day roughage was given in the form of paper pulp.

PARTURIENT HYPOCALCAEMIA PREVENTION IN COWS PRONE TO MILK FEVER BY DIETARY MEASURES

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Summary

Over 5 years (1967-1972) at Hoorn feeding measures in dry period and over the calving period intended to prevent low concentrations post partum of calcium were tested with about 170 parturient cows prone to milk fever. The trials examined influences of changes in Ca and P contents of the diet, a day of fasting, feeding below recommended requirements for energy and protein, infusions of ethylenediaminetetraacetate intravenously or intramuscularly and their affects on post partal concentrations of Ca, P and Mg in plasma and on packed cell volume. From the evaluation of results, dietary measures were designed that prevented low plasma levels of Ca in a trial with 45 cows prone to milk fever. These cows were fed to a Ca-poor diet (33.1-43.9 g/day) pre partum and a Ca-rich diet (148.3-196.8 g/day) post partum and adequate in energy, protein, P, Mg and vitamin D. No milk fever occurred and in only 4 of the cows did plasma decline below 7.5 mg/100 ml.

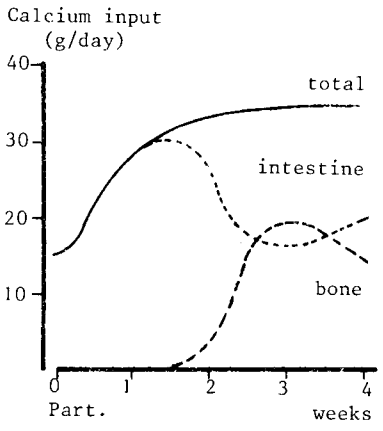
Recommended measures are as follows: (1) Provide a prepartal diet as low as possible in Ca ($\leq$ 0.50% Ca in dry matter); P and vitamin D intakes must be sufficient. (2) Just after calving, increase Ca-intake by an oral dose of 250 g $CaCO_3$ (100 g Ca) by bottle and increase Ca content of the diet ($>$ 1.0% Ca in dry matter); P and vitamin D intakes must be sufficient. (3) In the weeks pre- en post partum, provide an extra Mg of 30 g daily to prevent tetanic symptoms and low concentrations of Mg in plasma.

Introduction

To prevent hypocalcaemia the input and output of the available calcium pool must be in equilibrium.

The input comes from
1. absorption of intestinal calcium
2. resorption of bone calcium

The output goes to
1. fetus in the dry period
2. milk after calving
3. bone
4. faeces
5. urine



In slide 1 (fig. 1) changes are given in calcium input in response to the onset of lactation (from Ramberg and co-workers 1970). Increase of intestinal calcium absorption starts earlier than calcium removal from bone.

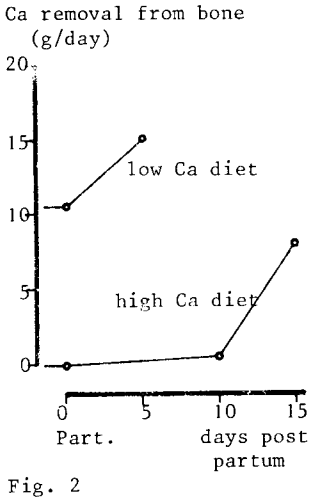


Fig. 2

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On a calcium-poor diet (slide 2)(fig. 2) calcium removal from bone after parturition increases earlier than on a calcium-rich diet (from Ramberg 1972). Obviously calcium metabolism is activated by a low calcium diet.

Although the calcium resorption from bone can be activated by a calcium-poor diet, in the first 72 hours after calving the absorption of intestinal calcium is still the main input of calcium needed for the sudden demand of calcium excreted with the colostrum. What can be done to get an activated calcium metabolism just before and during parturition ?

Two approaches can be followed in the dry period:

1. lower the input
2. increase the output

Trials

At Hoorn in 1967 we started with increasing the calcium output.

INFUSIONS OF EDTA (sodium-ethylene-diamine-tetraacetic-acid) binds plasma calcium in a complex. This complex is rather rapidly excreted with the urine.

This sudden output of calcium induces a decrease of blood calcium. A decrease in blood calcium stimulates the parathyroid glands i.e. parathormone production.

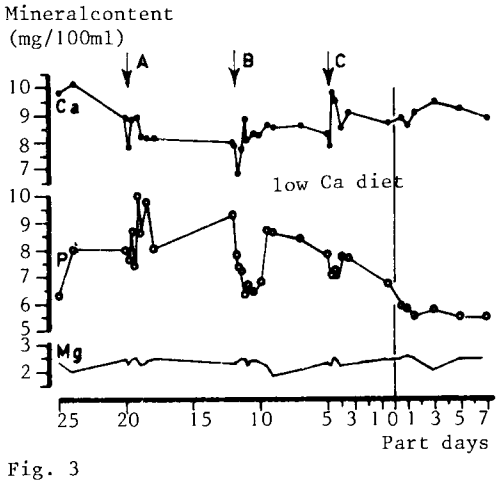

Fig. 3

Slide 3 (fig. 3). 3 Infusions of 50 mg EDTA/kg body weight intravenously were given each in 15 minutes. The reaction of blood calcium upon infusion C was different from A and B (more resistant to a sudden Ca-output).

The daily intake of calcium was 61 grams. Five days later the cow calved and no blood calcium decline was detected.

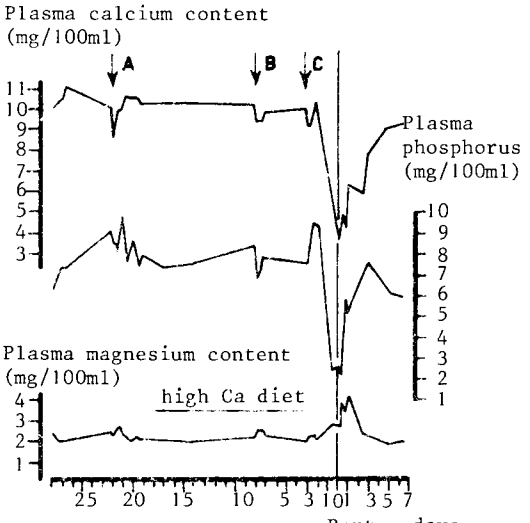

Fig. 4

Slide 4 (fig. 4) shows another experiment with 3 infusions of 50 mg EDTA/kg body weight. The last infusion was given 3 days pre partum.

The daily calcium intake was now 193 grams. In this case serious hypocalcaemia occurred with serious milk fever symptoms. The patient needed 4 calcium gluconate infusions to get normal again.

The difference between these two examples was as a main point the daily calcium intake.

It seems that the activating effect of EDTA can be broken down by a 3 days calcium-rich diet. This conclusion was supported by a field trial. In 15 milk fever prone cows two infusions were given at 4 weeks and about 2 weeks pre partum. 13 Control milk fever prone cows were not infused. Because of the rather calcium-rich diets in the dry period EDTA-INFUSIONS DID NOT GIVE ANY PROGRESS in milk fever prevention under farm circumstances.

Therefore we started to investigate the possibility in lowering the calcium intake with the diet fed during 6 weeks of the dry period.

During 5 years (1967-1972) we borrowed from local farmers about 170 milk fever prone cows. They were collected about 6 weeks pre partum, stalled at our experimental farm and send home about 2 weeks after calving.

FEEDING TRIALS

Every year there was a control group and an experimental group. Experimental group 1 received a Ca-poor diet for 5 weeks. The last week pre partum a Ca-rich diet was given (128 g/day). Experimental group 2 a Ca-poor and P-rich diet during the weeks pre- and post partum. Experimental groups 3 and 4 Ca-poor, P-normal diet pre partum and a Ca-rich, P-normal diet post partum was given. Besides that an extra dose of 100 g Ca as $CaCO_3$ was given just after calving.

RESULTS of the feeding trials.
The results are summarised in table 1.

Table 1. Influence of Ca- & P-intake on post partal blood calcium decline and milk fever incidence. All cows were completely milked soon after delivery.

	Controls cum.over 5 years	Experimental groups 1	2	3*	4*
Cows total	67	14	12	45	7
Ca-intake g/d	129	128	51	43	28
P-intake g/d	54	75	153	42	36
Blood Ca $\leq$ 7.5 mg/100 ml					
Cows	48	13	3	4	0
(%)	72	95	25	9	0
Milk fever					
Cows	18	7	1	0	0
(%)	27	50	8	0	0

* Just after calving 100 g Ca as an oral dose $CaCO_3$ was given and the concentrates changed from Ca-poor to Ca-rich

In another experiment we found that in the dry period an oral dose of 100 g Ca increases significantly blood calcium levels of cows on a Ca-poor diet (67 g/d) by 1.5 mg/100 ml, but not of cows on a Ca-rich diet (85 g/d).
This means that on a Ca-poor diet the higher absorption activity of calcium from the intestines pre partum can be used to achieve post partum an elevated blood calcium content. A slow adaptation of the intestinal Ca absorption is also described by Ramberg et al. (1970) and many farmers have noticed that milk fever occurs in particular within 3 days after calving.

From our work and from others, our conclusion is that calcium-poor diets in the dry period give a higher intestinal absorption and a higher bone turnover rate. BOTH are needed to prevent hypocalcaemia in parturient cows in general.
Therefore we recommend a calcium-poor diet in the dry period and a calcium-rich diet just after calving during at least 3 days.
Milk fever prone cows should be given an oral dose of 100 g Ca immediately after calving.
Magnesium in blood plasma of milk fever patients is often too low. An extra daily dose of 30 g magnesium can avoid this without inducing milk fever.
Although there are some aspects about calcium metabolism still unknown, the facts we know now give enough background to compose a practical advice in preventing parturient hypocalcaemia milk fever to the farmer.

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Summary of the discussion

Five questions were formulated. 1. The decline of post partal calcium contents in blood is a better indicator for the occurrence of milk fever than the usually observed symptoms. 2. In performing trials with dairy cows or herds it is important to define the calcium content of the ration pre partum. 3. The age of the cows most prone to milk fever is higher in the Netherlands than in Scandinavia. Has this anything to do with the calcium content of the feed? 4. Older cows can absorb calcium to a maximum of 70%, as found in Hoorn (see J. van Leeuwen in T. Diergeneesk. 1976). Don't we have to reconsider the calcium requirements of lactating and dry cows in the light of these observations? 5. It is known that overfeeding with caps and leaves of sugar beets induces rumen acidosis and hypocalcaemia at the same time. Overfeeding with concentrates produces acidosis; is it not needed to look also in this case for hypocalcaemia? Is there a connection to the effects of the barley diets in the experiments of Wittwer et al.?

THE COPPER METABOLISM IN MILK COWS: EXPERIMENTS WITH ^{67}Cu

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Introduction

Using electronic counting systems the liver of milk cows can be approached from the right side of the animal and the quantity of radioactive copper therein measured in vivo from a fixed location on the skin at the maximum counting level, usually over the 10th intercostal area. Injected ^{64}Cu isotope accumulated on the average 30% less in milk cows during the last three months term of pregnancy than otherwise. The liver of the fetus obviously competes for the circulating radiocopper in the maternal bloodstream (Binnerts, 1967). Similar injections with ^{64}Cu, intravenously or intramuscularly, revealed that grass fed cows accumulated on the average approx. 12% less radiocopper in their livers than cows fed hay and concentrates. The difference was greatest in spring and amounted to over 20%, whereas in autumn the difference was a 5% low. In order to measure the smaller differences of the order of 10% a more refined procedure has to be adopted. Pairs of milk cows, comparable as much as possible in body weight, age and milk yield were subjected to reversal feeding experiments. Each time two pairs were investigated in such a way that grass and hay could be fed simultaneously.

It would be agreeable if the differences in liver uptake could be expressed in weight quantities of copper, in stead of percentage dose, but the conversion is rather difficult; this will be elucidated elsewhere. Nevertheless, as a first approximation the copper fractions in the body of the same cow under grass or hay feeding may be assumed identical. This would result in 12% depression under grass feeding in total liver copper uptake. Probably the winter feed contains more copper than grass, so that the difference must be even somewhat larger. Since these results were obtained in experiments with intravenous or intramuscular injection, the effect will not be caused by absorption differences. It undoubtedly represents a fundamental disturbance in the intermediary copper metabolism.

The fate of the injected copper

Further study was undertaken as to the fate of the copper not going to the liver. In cows not much of the copper is lost in urine or milk, but the endogenous fecal loss is appreciable. A first report as to this loss was given in the preceeding Conference (Binnerts, 1973). In later experiments the absolute values differed, probably depending on the copper status of the animals, but it was felt that the short half life of ^{64}Cu made the results at 5 days and longer rather inaccurate, owing to the large correction factors for radioactive decay. Although ^{67}Cu is a well known isotope with a longer half life, it has to be made in a cyclotron, and the quantities usually were rather small. We have published recently (Binnerts et al., 1976) a method of production starting from zinc, that results in higher recoveries, of the order of a couple of a hundred microcuries, sufficient for experiments with 3-4 cows at a time. Again the radiocopper was injected. The isotope, after some study, could be made entirely pure, except for a large amount of ^{64}Cu activity at the onset, which declined to zero rather rapidly during the first week. The ^{64}Cu contamination served to monitor externally liver and injection sites, as was done in the previous experiments. The ^{67}Cu could be very effectively measured in se- and excretion products, including intestinal fluids. Table 1 gives a summary of the results of some of the results, expressed in percentage dose per day. They were obtained by radioactive counting, using large 3 l Marinelli beakers, and by atomic absorption measurement of chromium and ytterbium digestion markers (Binnerts and Boer, 1975).

During this reversal experiment the cows were in the isotope stalls with one cow getting weighed ratios of hay and concentrates as calculated according to milk production, and the other cow receiving frozen grass from one homogeneous lot, ad lib., vice versa. The table shows that grass feeding resulted in higher levels of radio copper excreted. How important these differences are, can only be judged when they can be expressed in weight quantities. Sampling in the intestinal tract for the ratio of radiocopper over total copper (specific activities, s.a.) is not appropriate, because of the large dilution with unabsorbed food copper. The only way seems to be analysis of the pure digestion juices and the other copper containing excretion materials, but pure preparations are hard to obtain. A reasonable alternative is analysis of blood, which seems justified in so far as the blood copper is the precursor of copper in the excreted materials. In Table 2 both the s.a. of the direct reacting copper fraction and of the

ceruloplasmin fraction have been used for the calculations.

Table 1. Excretion of radio copper (mean of 4 days)

Name of Animal	Type of feeding	Quantities in (% dose per day) Duodenum	Ileum	Feces
Dina 4	grass	5.90 ± 0.23	9.50 ± 0.41[1)]	6.60 ± 0.48[2)]
Dina 4	hay	5.24 ± 0.73	5.74 ± 0.60	5.02 ± 0.71
Anna 22	grass	8.55 ± 0.98	7.89 ± 0.57[3)]	10.69 ± 0.25[4)]
Anna 22	hay	6.24 ± 1.22	7.26 ± 0.60	9.76 ± 0.82

[1)] Difference $P < 0.01$ [2)] Difference $P < 0.05$

[3)] Re-entrant fistula blocked during part of one day

[4)] Period 2 days before and 2 days after the blocking date

Table 2. Average excretion of total copper (mean of 4 days)

Name of animal	Type of feeding	Quantities in (mg per day) Duodenum		Ileum		Feces	
Dina 4	grass	52.7[1)]	34.3[2)]	93.1[1)]	66.6[2)]	58.9[1)]	38.4[2)]
Dina 4	hay	52.4	37.8	57.4	41.3	50.2	36.1
Anna 22	grass	232	145	213	134	289	182
Anna 22	hay	145	86.7	170	101	274	136

[1)] Based on the s.a. of plasma ceruloplasmin

[2)] Based on s.a. of the direct reacting copper fraction in plasma

Compared to the calculated copper intakes, 170 and 220 mg/day for the two rations, these figures are large, especially in cow Anna 22.

Nevertheless, as a first approximation, accepting the specific activity values of the direct reacting copper of the same day the differential excretions could be calculated, as given in Table 3.

Table 3. Endogenous fecal copper losses (in mg/day)

		duod.	ileal	further
D 4	grass	+ 34	+32	-28
	hay + conc.	+ 38	+ 3	- 5
A22	grass	+145	-11	+44
	hay + conc.	+ 87	+14	+35

All these results are averages of four days of sampling with approx. 12 spot samples in hourly intervals taken per day. The effects therefore are not spurious ones, but they represent a systematic and continuous trend of the metabolism. In all cases the copper content in the proximal duodenum right after the passage through the stomach system is remarkably high. Possibly this has something to do with the known high copper content of the intestinal lining. Comar et al. (1947) for instance reported nearly three times the amount of radio copper accumulated in gastrointestinal mucosa, than in the corresponding muscular tissues. We have found copper contents well above 15 ppm in samples of freeze dried mucous obtained from the walls of the abomasum. Secondly, the excretion of copper with the bile seems to be rather unimportant, contrary to the experience with other animal species. That the bile is added between duodenal and ileal fistula was clearly shown by the colour of the obtained fluid. Backflow has not occurred to a considerable extent. Thirdly, in cow D4 it seems that approx. the same quantity of copper is reabsorbed from the further parts. It seems that this depends on the individual animal and/or the location of

the fistulae.

In further experiments with labelled caeruloplasmin (human caeruloplasmin, labelled by Dr. Van de Hamer at Delft) the total fecal excretion, although 2x as large as found in Table 1, remained too low to explain all excretion through this form. It therefore may be concluded that it was right not to use the specific activity values of caeruloplasmin in the above calculations. Maybe the caeruloplasmin still is important as a precursor in the large intestine, but certainly not in the entire system. Further experiments are in progress.

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Summary of the discussion

Direct reacting copper in blood plasma is the fraction binding to added carbamate reagent, in contrast to the copper in the metal protein (ceruloplasmin) fraction.

EFFECTS OF COPPER EDETATE INJECTION ON COPPER AND COPPER ENZYME STATUS OF BLOOD AND LIVER IN CATTLE AND ON THE MILK YIELD OF COPPER-DEFICIENT VERSUS TREATED COWS

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Summary

Four months after injection of copper edetate s/c, copper levels in the blood and liver of calves and cows were higher ($p < .001$) in treated than in control animals. Treated animals also had higher activities of caeruloplasmin in serum and plasma ($p < .001$) and of cytochrome oxidase in liver ($p < .01$ to $p < .001$) than those of control animals.

Individual milk records were analysed for 7 occasions between May and September in treated versus control cows in 13 herds. In all, records from over 700 cows were analysed. Controls in all 13 herds were deficient in copper. In August, 4 months after injection, treated cows had 40.4 ppm copper in liver DM, as compared with 9.7 ppm in controls ($p < .001$). Despite the biochemical response, treatment had little effect on milk yield. The overall mean increase in yield in treated versus control cows was 0.39 kg/cow/day. This increase was not significant ($t = 1.53$; $df = 717$). Only one herd out of 13 showed a significant yield response to treatment (1.5 kg/cow/day; $t = 2.05$; $df = 61$; significant at $p < .05$).

Low copper status in the animal need not cause production loss. Factors which may possibly cause hypocuprosis are discussed.

Introduction

During the late 1960's we knew from soil surveys and analysis of samples from problem farms that molybdenum excess was widespread and was associated with low copper status in cattle. Although some studies in this country had indicated that copper supplementation caused greatly increased weight gain in young cattle (Poole, 1963, 1973; Rogers, 1970, 1971; Poole et al., 1974)other studies indicated little or no effect in calves and, especially, 1-2 year old cattle (Todd et al., 1967; Poole, 1963, 1968, 1969, 1974; Poole et al., 1974).

Controlled studies were planned for 1971 - 1973 to assess the practical results of copper supplementation in the production of young cattle, mainly in known high-molybdenum areas. A number of known or suspected normal herds were included. As part of this study, the effects of copper edetate injection on tissue chemistry were monitored in control and treated calves.

In the summer of 1973, outbreaks of suspected hypocuprosis were reported from many farms in two areas in which we had not seen this problem previously. The clinical picture included: scouring, infertility, unsatisfactory milk yields, non-specific abortions, stillbirths or weak calves. Blood tests confirmed that herd copper status in both of these areas was very low. Soil molybdenum was high and herbage molybdenum ranged from 5-120 ppm DM. It is not certain that all of the clinical signs were due to the molybdenum-induced copper deficiency. Injection of half of the cows on one of these farms with copper edetate in late autumn 1973 increased the milk yield in treated cows by about 1.25 kg/day. Yields in both treated and control groups were low, as the end of their lactation was near (Rogers, 1973).

Controlled studies were planned for 1974 - 1975 to assess the effect of copper edetate injections on milk yield in these areas.

Materials and methods

Animals

Calves were weighed in the spring of 1971, 1972 and 1973 and were allocated at random (within sex, within herd) to treatment or control groups. In all, 92 herds were studied (37, 35 and 20 in the 3 years respectively). Copper (100 mg), as edetate, (Glaxo: Coprin) was injected s/c into the treatment groups after weighing. Most of the herds studied were of beef suckler calves at grass with their dams. Samples of blood and liver tissue were taken from 5 treated and 5 control calves on each farm about 4 months after treatment.

Cows on 8 farms, covering the two problem areas, were allocated at random (within herd, within "heifer" or "cow" groups) into treatment or control groups in 1974. Copper (200 mg) as edetate was injected in spring and late summer. In 1975, 5 herds remained in the trial. All milking cows remained in the same groups as in the previous year. Replacement heifers were randomized into treatment or control groups. The copper was injected in 1975 in late winter, spring and late summer. During the trial, all cows were at grass, without concentrate feeds. Samples of blood and liver tissue were taken from 6 treated and 6 control cows in each herd (total 13 herds) about 4 months after the

spring treatment.

Milk recording for individual cows was to be done fortnightly. However, the herd owners did not record as frequently as we had hoped. The number of full recordings in each lactation ranged from 7-12, beginning in May or June. Calving was mainly in February, March, April, so that on average, cows were calved about $2\frac{1}{2}$ months at the start of recording.

Analytical methods

Blood samples were collected from the jugular vein in heparin containers and also in glass tubes containing no anticoagulant.

Liver biopsies were taken under local anaesthesia from the second-last or last intercostal space. (Only once in 106 farm visits was permission to biopsy the animals refused by the owner). The samples were stored in glass tubes in flasks cooled by solid CO_2 until return to the laboratory. There, they were stored in deep freeze until required for analysis.

The copper content of blood and liver samples was determined by AA spectrophotometry after digestion in a mixture of sulphuric : perchloric and nitric acids.

The activity of caeruloplasmin in serum or plasma was determined after incubation in buffered paraphenylenediamine for 35 minutes. The reaction was stopped with azide. Optical density at 530 nm was read in a Gilford 300N spectrophotometer.

The activity of cytochrome oxidase in liver was assayed by kinetic oxidation of reduced cytochrome C (Boehringer Corp.) according to the method used by Poole, 1973.

The analyses were done by the staff of the Analytical Services Laboratory and Field Investigations Dept., Agricultural Institute, Dunsinea.

Statistical methods

The effect of copper injection on tissue chemistry was examined by within-farm and within-year Student's t test on treatment versus control means. In Table 3, the treatment effect was also examined over the combined data of 1974 - 1975.

The milk yield data were analysed by John Sherrington, Statistics Dept., Agricultural Institute, Dublin 4. The data were reduced to analysis of seven recordings per farm per year. This was required, as seven recordings was the lowest common denominator between farms. The recordings covered a variable period of 90 to 144 days, between May and September. For analysis, records for over 700 cows on 13 farms were examined.

Milk yield data were analysed in three ways.

(a) Model: $Y = X + \text{Treatment} + \text{Lactation No.} + \text{Herd} \pm \text{Year} + b_1$ (interval calving to recording) + b_2 (interval January 1 to recording) + Error, where Y = actual yield on any one of the 7 recordings. The equation was solved for X and analysis of variance was done.

(b) Unadjusted values of Y were examined for treatment effects using Student's "t test" "within-herd, within-year" and over the "13 farm-years".

(c) The mean yield between the first and seventh recording (inclusive) was calculated, adjusting only for the intervals between recording. These means + SE were calculated for treatment and control groups "within-herd, within-year" and also over the "13 farm-years." These are shown in Table 2.

Results and discussion

Effects of copper on tissue chemistry

In the calf trials (1971-1973), some herds known or suspected to have normal copper status were included. Herbage analysis during these trials showed yearly farm averages for copper and molybdenum ranging from 6-12 ppm DM and 1-15 ppm DM respectively.

In contrast, the cow herds (1974-1975) had been selected because of known hypocupraemia and high area molybdenum status. Herbage analysis in 1974 (not done in 1975) showed yearly farm averages for copper and molybdenum ranging from 7-10 and 4-18 ppm DM respectively.

The difference in selection procedure between the calf and cow trials (and of the molybdenum backgrounds between the trials) largely explains the higher annual mean liver copper levels in the calf controls (22.5 - 37.4 ppm DM) as compared with those in the cow controls (9.1 - 10.0 ppm DM). See Table 1.

Table 1 summarises the tissue chemistry in treated and control animals 4 months after treatment. All parameters were higher ($p < .001$) in treated than in control animals except cytochrome oxidase in liver; in 2/5 years this difference was significant at $p < .001$, in the remaining 3 years at $p < .01$. Response in tissue copper or copper enzyme varied widely between farms. On some farms, usually those with very deficient controls, the increase in liver copper in treated animals had disappeared in less than 4 months. Table 2 shows the tissue chemistry for treated and control animals and the effect of treatment (T-C) at 4 months after injection. It can be seen from the table that herds whose controls had a normal or marginal level of liver copper (> 20 ppm DM) responded markedly in liver copper but to a much lesser extent in cytochrome oxidase, blood copper or serum caeruloplasmin. Deficient or very deficient herds gave greater responses in the latter three parameters.

Effect of copper on milk yield

Table 3 shows the mean milk yield for treated and control cows on each farm calculated by method (c) above. Also shown are the mean liver copper levels in August (c. 4 months after treatment) in treated and control cows. The treatment effects and their significance are shown. All methods of analysis indicated a positive but not significant effect on yield, despite a highly significant effect on tissue chemistry. Method (c) indicated an increase of 0.39 kg milk/cow/day ($t = 1.71$; NS) overall. It also indicated that only 1 farm in 13 farm-years gave a significant ($p < .05$) response (an increase of 1.5 kg/cow/day). This effect could be explained by the chance hypothesis ($p > .05$) in terms of 1/13.

General discussion

There is no doubt that hypocupraemia can be associated with severe clinical signs (hypocuprosis) and production loss in calves, older cattle and adult cows. Copper therapy can prevent or treat the condition. This has been reported extensively in the international literature and many well documented descriptions of clinical copper deficiency in Ireland have been published. In addition, veterinary surgeons in practice and in the regional veterinary diagnostic laboratories, encounter various syndromes which appear to respond to copper.

However, failure of copper injection to increase milk yields significantly in treated cows as compared with copper-deficient cows has been reported previously in this country (Poole and Walshe, 1970) and in New Zealand (Goold and Smith, 1975). In both of these instances a good biochemical response was obtained. The present study confirms these findings, on a larger scale.

Todd et al., (1967) reported a non-clinical hypocupraemia in suckler calves and the failure of copper supplement to improve weight gain in treated calves. Other studies also reported little or no weight response to copper in deficient young stock and, especially, older cattle (Poole, 1963, 1968, 1969, 1973; Poole et al., 1974; McCarthy et al., 1972; Rogers et al., unpublished).

The combination of clinical observation and applied research indicates that unknown factors may affect the copper deficient animal to cause production loss. These factors could include various "stresses" such as high production, high stocking rates, parasitism, nutritional or other deficiency states, intercurrent disease, genetic predisposition, the presence of antagonistic or toxic factors, sparing effects by other minerals or compounds etc. Assessment of the likelihood of production loss in any given herd is further complicated by the method of defining the degree of deficiency: by blood test, enzyme test, liver test. It is difficult to define the normal levels and to find critical levels below which hypocuprosis will inevitably occur. At low levels the biological state of tissue copper may be more important than its absolute levels.

Hypocupraemia per se need not cause obvious ill-health unless other factors are also present. A full understanding of the role of copper deficiency in production disease will depend on identification of the unknown factors.

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Summary of the discussion

It was suggested that simultaneously occurring deficiencies of other parameters might have influenced the results in such a way as to mask the effects of copper treatment in copper deficient cows. It is however, not known from literature what to expect under these conditions.

Table 1. Mean levels of liver copper, liver cytochrome oxidase, serum and plasma caeruloplasmin, and whole blood copper in treated and control animals, 4 months after treatment. The differences(T - C) are all significant at $p < .001$, except where indicated (** = $p < .01$).

Experiment		Total no. sampled (a)	Liv Cu (ppm DM)		Liv Cyt Ox (uM/min/g wet)		SCPA (OD units)		PCPA (OD units)		Blood Cu (mg/100 ml)	
			T	C	T	C	T	C	T	C	T	C
Calves	1971	343-375	68.5	37.4	12.2**	10.4	.099	.065	-	-	.090	.069
	1972	352	52.1	22.5	13.8	11.1	.119	.072	.186	.111	.070	.052
	1973	190-204	71.3	28.4	21.5**	17.9	.099	.071	.161	.114	.069	.059
Cows	1974	96	47.2	10.0	19.3**	15.1	.140	.073	.177	.093	.062	.042
	1975	60	26.7	9.1	16.9	8.1	.089	.029	-	-	-	-

(a) This table may be used to examine treatment effects but not the relationship between the different parameters, as the (n) values varied in the range shown and the analytical technique for CPA was improved in 1972

Table 2. Tissue chemistry (a) 4 months after treatment in treated and control animals, as a function of the degree of copper deficiency in control groups

Mean liver copper (ppm DM) in control groups		Liv Cu			Liv Cyt Ox			Blood Cu			SCPA		
		T	C	(T-C)	T	C	(T-C)	T	C	(T-C)	T	C	(T-C)
< 10	(calves)	24.9	6.7	18.2***	12.12	7.60	4.52***	.0658	.0405	.0253***	.0883	.0344	.0539**
	(cows)	27.6	8.6	19.0*	12.94	8.34	4.60*	.0607	.0350	.0257***	.1100	.0349	.0751**
10 - 20	(calves)	52.7	13.9	38.8***	17.33	14.51	2.82***	.0828	.0628	.0200***	.1136	.0783	.0398**
	(cows)	58.0	11.4	46.7*	18.64	15.00	3.64*	.0644	.0535	.0109*	.1364	.0894	.0470*
20 - 30	(calves)	81.6	25.3	56.3***	14.45	13.84	0.61 NS	.0892	.0759	.0133**	.1217	.0939	.0278**
> 30	(calves)	118.8	72.5	46.3***	16.77	16.52	0.25 NS	.0855	.0810	.0045*	.1204	.1047	.0157*

(a) See footnote to Table 1 and Table 3

Table 3. Milk yield (kg/day) and liver copper (ppm DM) in treated (T) and control (C) cows. The significance of the difference (T-C) between groups is shown.

Farm and Year	Milk Yield		Liver Copper		Farm and Year	Milk Yield		Liver Copper	
	T	C	T	C		T	C	T	C
OB 74	10.2 NS	10.2	10.6*	6.9	OB 75	11.9 NS	12.5	15.1 NS	7.6
OC 74	10.5 NS	10.1	18.4*	9.2	OC 75	12.4 NS	10.3	27.1*	10.5
OG 74	9.8 NS	9.6	47.0**	11.1	OG 75	11.0 NS	10.6	53.2*	9.5
Q 74	11.3 NS	10.2	36.2**	10.0	Q 75	12.8 NS	12.5	29.1**	10.7
C 74	11.2 NS	10.9	13.8 NS	9.8	C 75	11.9*	10.4	24.5**	7.2
B 74	11.6 NS	11.3	112.9***	13.1					
OF 74	14.1 NS	14.0	64.5*	8.4					
F 74	9.8 NS	10.9	74.3**	11.6					
					74/75 TOTAL	11.42NS	11.03	40.5***	9.7

NS = not significant; *, **, *** = significant at $p < .05, .01, .001$ respectively

GLUTATHIONE PEROXIDASE IN RUMINANTS AND SUSCEPTIBILITY TO NUTRITIONAL MYOPATHY

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Summary

The correlations between erythrocyte glutathione peroxidase activities and whole blood selenium concentrations in cattle and sheep are described and responses of these two parameters in cattle to a low selenium diet and selenium injection illustrated. Glutathione peroxidase activities of ten specified muscles in cattle on the selenium deficient diet were compared with those in similar cattle injected with selenium. Nutritional myopathy has been increasingly common in England and Wales during the past five years and the possible role of selenium availability is discussed. Preliminary results are presented of a survey of biological selenium-status in sheep.

Introduction

Nutritional myodegeneration, an acute disease of cattle and sheep, has been described in many countries and vitamin E and selenium deficiences are known to be associated with this disease (Blaxter, 1962). The enzyme glutathione peroxidase (E.C. 1.11.1.9) obtained from erythrocytes has been shown to contain 4 atoms of selenium per molecule of enzyme in cattle (Flohe, Günzler and Schock, 1973) and sheep (Oh, Ganther and Hoekstra, 1974). Hoekstra (1975) has proposed a mechanism by which cells subjected to vitamin E and selenium deficiency become susceptible to the damaging effects of lipid peroxides, glutathione peroxidase normally protecting against such damage.

The apparent increase in nutritional myodegeneration, particularly in yearling cattle, in Britain (Anderson, Berrett and Patterson, 1976) may be due to the increasing use of selenium-deficient home-grown feedstuffs (Bradley, 1976). In this paper some selenium deficient areas of England and Wales are identified using the glutathione peroxidase activity of sheep erythrocytes as an indicator of biological selenium-status.

Results and discussion

Experiment 1

Erythrocyte glutathione peroxidase activities and whole blood selenium concentrations were determined in 191 cattle and 43 sheep. The correlation coefficients were +0.838 and +0.970 for cattle and sheep respectively, both being significant at the $P < 0.001$ level.

Experiment 2

Fifteen cattle were fed a diet containing 0.01μg/g of selenium and five of these were injected subcutaneously on five occasions at 2 week intervals with 2.5 mg of selenium as sodium selenite. All cattle were bled weekly. Thirteen cattle were killed (9 uninjected and 4 injected) and the glutathione peroxidase activity assayed in the following muscles; biceps femoris (proximal end), semimembranosus, middle gluteal, supraspinatous, infraspinatous, triceps brachii, ulnaris lateralis, the crura of the diaphragm, the masseter and the heart. Figures 1a and b show the changes in erythrocyte glutathione peroxidase activities and whole blood selenium concentrations respectively in the uninjected and injected groups over a 10-week period.

Figure 1a and b. Changes in the mean erythrocyte glutathione peroxidase activities and whole blood selenium concentrations.

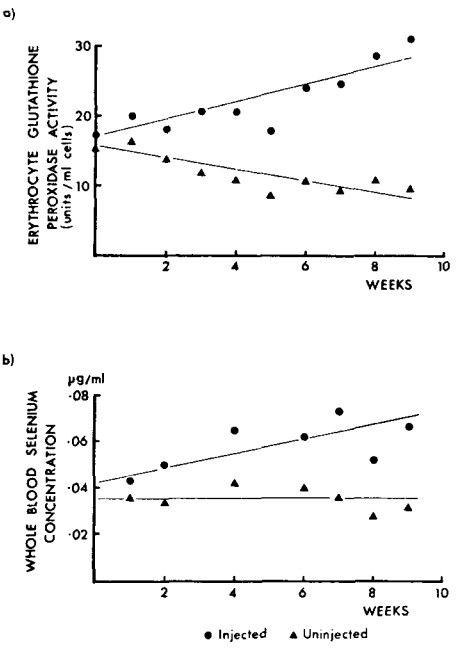

Table 1 shows the geometric means of the muscle glutathione peroxidase activities of the 9 uninjected and 4 injected cattle and the ratio of these two means.

Table 1. The glutathione peroxidase activities of ten muscles from 9 cattle on a selenium-deficient diet compared with those from 4 cattle receiving five injections of 2.5 mg selenium at 2 weekly intervals.

	Glutathione peroxidase activity geometric mean		Ratio of means Injected: Uninjected
	Uninject.	Inject.	
Biceps fem.	169	1880	11
Semimem.	9	757	85
M. gluteal	16	1080	66
Supraspin.	102	1300	13
Infraspin.	224	1770	7.9
Triceps	29	1120	38
Ulnaris lat.	187	1230	6.6
Masseter	899	6420	7.1
Diaphragm	359	3080	8.6
Heart	1390	8010	5.8

Experiment 3

Heparinized blood samples were obtained from groups of 20 sheep grazing at numerous locations throughout England and Wales. All the sheep had been grazing at one location for at least 2 months, from which it was assumed that the erythrocyte glutathione peroxidase activities were a reflection of the selenium availability to sheep at that location. Erythrocyte glutathione peroxidase activities were determined and the mean and standard error calculated. The biological selenium-status equivalent to the means was calculated from the correlation for sheep in experiment 1, and Figure 2 illustrates the distribution of these selenium 'values' in sheep over England and Wales.

Allen, et al (1975), Boyd (1975) and Thompson, McMurray and Blanchflower (1976) demonstrated a correlation between erythrocyte glutathione peroxidase activities and whole blood selenium concentration in cattle and the latter authors also in sheep. These correlations have been confirmed by the present study and it is evident that erythrocyte glutathione peroxidase is a reliable indicator of biological selenium-status, where this is a reflection of the overall selenium intake in the preceding two or three months. However, it is evident from Table 1 that glutathione peroxidase in cattle muscle, the target tissue of nutritional myopathy, is also affected by the selenium intake, muscles of selenium-injected cattle having between 6 and 80 times as much enzyme as those from uninjected cattle. It is also evident that muscles commonly affected in nutritional myopathy of the yearling, the locomotor muscles, have less activity than the heart, masseter and diaphragm which are rarely affected in the yearling. Indeed in some locomotor muscles of selenium-deprived cattle the activity was undetectable, but it was in this group of muscles that response to selenium injection was the greatest.

Figure 2. The mean biological selenium-status of sheep in England and Wales: * low (< 0.05 μg/ml), marginal ♦ (0.05-0.1 μg/ml) and adequate ▲ (> 0.1 μg/ml) determined from the correlation obtained in experiment 1.

Figures 1a and b show that the erythrocyte glutathione peroxidase activity in cattle on a low selenium intake steadily declines, but this can be more than compensated for by the subcutaneous injection of selenium every two weeks. Blood selenium levels increased in

the cattle given selenium by injection but no detectable change occurred in the blood selenium concentration in the cattle put onto a low selenium diet.

Biological selenium-status of sheep so far determined in England and Wales (as shown in Figure 2) indicates that there are many areas where the availability of selenium is inadequate or marginal. The selenium concentration in proprietary protein concentrate, derived from soya bean or fish meal, is usually high and milking cows on an appreciable quantity of this feedstuff have an adequate selenium-status. However, cattle fed principally on locally-grown feeds such as barley, and hay or straw with little or no additional protein, may be subject to an inadequate selenium intake. Urea as a non-protein nitrogen feed will also seriously reduce the selenium intake. There has been a trend in Britain over the past 5 years to omit proprietary protein concentrates from the diet of store and fattening cattle, and rations for the winter have been provided from home-grown cereal and hay or straw. Such a diet may be nutritionally adequate with respect to metabolizable energy and protein, and cattle may continue to grow satisfactorily but it may be selenium deficient. This change in feed management may partly explain the increase in the now commonly recognized selenium-responsive disease, nutritional myopathy.

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Summary of the discussion

Plasma glutathione peroxidase was not used in this investigation, because of its extremely low values in cattle. The method is easy and cheap when developed for automation. Kits are not yet available and duplo differences are reasonably small. In the Netherlands (Binnerts) similar determinations, coupled with selenium analysis in milk, have revealed that the area low in Se is identical to the goiter area, with the exception that the river clay area is high in selenium, and low in iodine. Sand and peat subsoils are low in both Se and I.

NUTRITIONAL MYODEGENERATION (WHITE MUSCLE DISEASE) OF YEARLING AND ADULT CATTLE

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Summary

In the United Kingdom there is a reported increase in nutritional myodegeneration in yearling, young adult and pregnant cattle. Most of the investigated outbreaks have been associated with proven nutritional deficiencies of vitamin E and selenium. Some deficient diets have resulted from the use of unsupplemented urea and propionic acid-treated cereals.

Pathologically the disease is a skeletal myodegeneration sometimes accompanied by myoglobinuria. Type I fibres are preferentially diseased. Histochemical profiles are altered following exercise or myodegeneration. The M. biceps femoris is a primary target muscle and, as it forms part of the rump steak, meat from affected animals killed for beef before regeneration is complete may be susceptible to post mortem quality changes.

In unprepared animals clinical disease, which is accompanied by a raised plasma creatine phosphokinase (CPK, E.C.2.7.3.2.) activity in the acute phase, is often precipitated by sudden increase in exercise and sudden exposure to inclement weather. A period of acclimatisation before turnout is therefore recommended.

Introduction

Myopathies of cattle associated with nutritional deficiencies of vitamin E and selenium are well recorded in many different parts of the world (Hadlow, 1973). The pathology of the condition is documented by Hadlow (1962), Hulland (1970) and Bradley (1975) and is characterised by skeletal or cardiac myodegeneration or both. Until the last few years the great majority of papers on the subject have related to the disease in young cattle up to about six months of age. However, more recently, a similar disease has been recorded in yearling and adult cattle by Doig (1970), Christl (1971) and Allen et al. (1975) and in pregnant heifers by Van Dreumel (1975).

Economic pressures have induced a search for cheaper effective rations for cattle. These have included urea as a source of nitrogen and propionic acid-treated maize or barley. As urea contains no selenium and propionic acid-treated cereals lose much of their vitamin E content in a short time as a result of the acid treatment (Allen et al., 1974), induced nutritional deficiencies may result from the use of these unsupplemented feedstuffs. Cattle may then be susceptible to myodegeneration when they are subjected to the stress of inclement weather and sudden increase of exercise as when they are turned to pasture in the Spring after Winter housing (Allen et al., 1975). The purpose of this paper is to report some of the pathological findings in field and experimentally produced disease in adult and yearling cattle, to show how the dynamic nature of skeletal muscle should be exploited and to point out some potential dangers for the meat industry.

Materials and methods

Four vitamin E and selenium-deficient yearling cattle (from the field) with WMD were killed sequentially at three, six, nine and forty nine days after the onset of clinical disease and subjected to extensive post mortem examinations.

One vitamin E and selenium-deficient pregnant dry cow and fetus of 268 days gestation was killed 42 days after the onset of clinical disease and both were subjected to extensive post mortem examinations. X-radiographs of fetal long bones were taken for pathological study.

Twenty seven calves were experimentally maintained on a vitamin E and selenium - deficient diet. These were divided into two groups which were turned to pasture in inclement weather at different times in the Autumn. Four normally fed animals were injected with a selenium and vitamin E preparation eight days before turnout with the second group. Plasma CPK levels from all these and control animals kept indoors were monitored daily. Animals with raised or normal levels were subjected to detailed post mortem examinations at intervals after turnout. The survivors from these two groups were re-housed and maintained on the deficient diet. Five animals were injected five times at fortnightly intervals with a vitamin E and selenium preparation before turnout of the whole group in the Spring when similar biochemical and pathological studies were undertaken.

Pathological study centred on the skeletal musculature of all these animals, and up to 50 muscles from each animal were examined by standard histopathological, ultrastructural and histochemical techniques. Frozen sections were reacted for myosin ATPase after

pre-incubation at pH 10.4 and for succinic dehydrogenase or NADH tetrazolium reductase. The proportion of diseased, high, intermediate and low reacting cells for particular muscles were calculated and compared between muscles and between animals at different intervals after the onset of disease.

The vitamin E and selenium status of the diet, blood and tissues of representative animals were determined by the Biochemistry Department as were the plasma CPK levels in all animals after the onset of disease and in the experimental animals only, before turnout and before the onset of disease.

Results and discussion

During the last four years in the United Kingdom there has been a progressive increase in the reported incidence of nutritional myodegeneration in older cattle. This has been particularly apparent in yearlings and young adults (including bulls reared for beef) but also in dairy cows. Such cases have often been associated with unconventional feeding methods including unsupplemented diets based on straw, urea and propionic acid-treated cereals. Even if mineral supplements are provided these may not contain adequate quantities of selenium or vitamin E and they may not be fed in a way to ensure that adequate quantities are eaten. An additional stress factor associated with the onset of clinical disease in pregnant cows is a sudden alteration to the amount of food fed or concurrent disease (Gitter, Bradley and Pepper, 1976).

The clinical features of the adult disease are mainly locomotor disturbances resulting from non-fatal skeletal myodegeneration and sometimes myoglobinuria whereas in the calf myoglobinuria is seldom recognised and cardiac myodegeneration may occur alone or as a frequent though inconstant accompaniment.

To date fetuses from clinically affected and nutritionally deficient cattle have shown no evidence of myodegeneration (Van Dreumel 1975 made similar observations in fetuses from affected pregnant heifers in Canada) but in one of our calves there was radiographic evidence of growth arrest lines in long bones at intervals from 203 days of gestation.

The onset of clinical signs in all animals is accompanied by a rise in plasma CPK which is a valuable diagnostic indicator in the early stages of the disease.

In the field, clinical recovery usually occurs following the removal of the stress factors, improvements and correction of the diet and/or following injections of therapeutic doses of vitamin E and selenium.

The pathological feature of the disease is skeletal myodegeneration which occurs at a time coincident with the raised plasma CPK level. This phase is rapidly followed by phagocytosis and regeneration and a fall in plasma CPK activity.

Histochemical study of the frozen sections from the M. semitendinosus and M. gluteus medius of normal and diseased animals which had been reacted for myosin ATPase after pre-incubation at pH 10.4 has shown that type I (slow aerobic) fibres are five to seven times as likely to be diseased as type II (fast anaerobic) fibres when the severity of affection was moderate. When muscles were severely damaged type I and type II cells could be totally destroyed over a wide area. These findings concur with those of Ruth and Van Vleet (1974) who showed a similar susceptibility to disease of type I cells in selenium and vitamin E deficient pigs with skeletal myodegeneration.

Experimental reproduction of a mild form of the field disease in yearling cattle has been successfully achieved at Weybridge by subjecting vitamin E- and selenium-deficient animals to the stress of turnout in inclement weather. A histochemical study of muscles from deficient and supplemented animals at intervals following turnout indicated that skeletal muscle is a dynamic structure, its muscle cells adapting themselves to the functional demands placed upon them by environmental conditions. The adaptation involves changes in fibre size (atrophy and hypertrophy) and changes in the histochemical profiles which principally involve changes in the means of producing energy (ie the aerobic and anaerobic capacity). Important factors initiating these changes are the sudden increase in exercise which follows turnout after winter housing and the effect of cold and wind chill. In the absence of skeletal myodegeneration there is a net increase in the aerobic capacity of the M. biceps femoris as revealed by histochemical methods.

In diseased animals a histochemical study of the M. biceps femoris one month after turnout showed that when the diseased muscle regenerates its histochemical profile is altered from what it was before degeneration.

Following turnout of deficient and supplemented experimental yearling cattle there was a biphasic rise in plasma CPK levels in many animals (For details see Anderson et al., 1976). The first rise occurs on the day following turnout and returns to normal on the following days. It is associated with myodegenerative changes in the M. ulnaris lateralis, Mm. flexores digitorum profundus et superficialis of the fore limb. The second rise occurs in the ensuing two weeks and is associated with myodegeneration of the proximal part of the M. biceps femoris which is considered to be a primary target muscle. This part of the M. biceps femoris forms part of the rump of beef (rump steak) which is a high priced cut. Since the disease is occuring in older cattle than hitherto at a time when cattle are close to slaughter weight

there could be important repercussions if animals were slaughtered before regeneration was complete since:-
a) the lesion is recognisable grossly as white streaks or patches in the muscle at the time of degeneration and shortly afterwards,
b) the muscle histochemistry is altered from normal at least until one month after the onset of clinical symptoms and
c) final pH (24hours post mortem) was 6.10 in a diseased animals M. biceps femoris killed one day after the onset of clinical signs compared with pH 5.55 in a normal animal. These features may render meat more susceptible to post mortem quality changes. So far there have been no reports of such problems from the meat trade but the possibility of its occurrence and detection should be recognised. WMD of older cattle is being increasingly diagnosed and if a correlation between it and the meat qualities of rump steak were proved it would suggest that production disease has advanced ahead of the knowledge needed to control it. This may also be true of recent changes in cattle feeding brought about by economic pressures. The greater reliance now being placed on home-grown as distinct from imported feed for cattle is exposing unsuspected selenium-deficient areas in the United Kingdom. The use of un-supplemented urea-based diets and of propionic acid treated cereals are added hazards.

It should also be recognised that the dynamic nature of muscle which allows it to adapt to the changing functional demands of the environment is an asset to be exploited rather than ignored. The changes in muscle metabolism which occur take time to effect and therefore sudden environmental changes should be avoided. Thus a period of acclimatisation before turnout is a simple recommended procedure which may prepare the animals better for any subsequent stresses.

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Summary of the discussion

Usually the more superficial muscles are found to be the most degenerated. A cold environment, resulting in reflex vasoconstriction, might depress the blood circulation in muscle. On the other hand, shivering, and similar counter reflexes to cold, will burn up some glucose, resulting in more stress to the already deficient oxidation system. Ranking of bovine muscles in terms of the proportion of muscle cell types did not show any corresponding disease susceptibility. In experimental disease the proximal biceps femoris is the target muscle. Selenium content in these separate muscles or muscle types has not yet been measured.

PALE, SOFT AND EXUDATIVE (PSE) MEAT, STRESS-SUSCEPTIBILITY (SS) AND THE MALIGNANT HYPERTHERMIA SYNDROME (MHS)

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Summary

Within the frame work of the Conference on Production Disease in Farm Animals the subject Pale, Soft and Exudative Meat with all its consequences certainly deserves attention. Selection for meat production in pigs has lead to problems with meat quality. These problems have been extensively discussed in two symposia around this subject in Zeist, The Netherlands in 1969 and 1971 and in Wisconsin, U.S.A. in 1972. Apart from the assessment of different meat quality parameters the attention was drawn to the relationship between meat quality, muscle biochemistry and the endocrinological state of the live animal. The condition of the muscle at the moment of slaughter plays a key role in the development of PSE meat. Evidence exists that PSE prone strains or breeds of pigs produce a malignant hyperthermia syndrome c.q. porcine stress syndrome when subjected to stress. The same reaction can be provoked by application of halothane. These phenomena were intensively studied the years after these meetings. By way of blood creatine kinase activity determination, bloodgrouptyping and halothane sensitivity test it looks that it seems to be possible not only to identify PSE prone pigs, but also to use these criteria effectively in breeding programmes. Now possibilities exist to develop meatier pigs without running into PSE meat problems. The conclusion is that certain production diseases can be effectively exterminated.

Introduction

In this conference over production diseases the PSE condition after slaughter and related subjects are the ones which will be presented and discussed on the first day. This implies that the PSE problem is regarded as belonging to the so-called production diseases.

Pale coloured, Soft and Exudative meat and the related problem Dark, Firm and Dry (DFD) meat is an unacceptable product of modern pig production. One might consider it as a post mortem phenomenon of a metabolic disorder in the live animal. We will try to develop this hypothesis in this introduction.

Previous work

Over the past twenty years much attention is paid by research workers all over the world to this meat quality defect.

In 1969 (1), 1971 (2) and 1972 (3) special symposiums have been organized in The Netherlands and in Wisconsin on this subject. In the proceedings of those conferences a wealth of information is given about all possible aspects of this problem.

Let us briefly summarize the most important trends.

Muscle

Post mortem muscle with a rapid metabolism i.e. rapid pH fall, rapid onset of rigor mortis produce most likely PSE. Also one of the typical characteristics is an increased temperature level. This accelerated glycolysis and breakdown of high energy phosphates is only partly due to the post mortem circumstances such as slaughtertime and slow cooling rate. Especially the deviated ante mortem muscle processes induce this meat quality development.

This hypothesis could only be tested with the different methods which were developed for assessing objectively the meat quality (pH, rigor and temperature measurement, transmission value method, colour and reflectance measurement).

This post mortem feedback gave us clear evidence that the equilibrium of vital muscle processes is much more weaker in PSE prone pigs. Accumulation of lactic acid and other intermediates of the anaerobic glycolysis together with low levels of high energy phosphates can be brought back to malfunctional structural elements such as sarcoplasmic reticulem, mitochondria and a relatively higher rate of muscle fibers geared for anaerobic glycolysis. This brings us to the conclusion that PSE can be regarded as a symptom of a dis-equilibrium of the muscle system. The question remains to which extent in the body other systems besides the muscle system were involved.

Other systems

The fact that so-called stress prone pigs which die very easily show very often PSE doesn't mean that beforehand between these two a close relationship should exist. One

should always bear in mind that animals which die suddenly not always are slaughtered in the normal way. The observed PSE-like meat might be caused by this abnormal slaughterprocess. In these animals several typical clinical symptoms have been observed such as blotching of the skin, trembling of muscle especially those of the legs and the tail and a high body temperature (Topel, 1969). The existance of breed differences suggest that those animals selected for muscle quantity are rather susceptible to sudden environmental changes.

Much work has also be done to elaborate in this context the function of the adrenals (Bouman, 1969) and the thyroid. The metabolic rate of the cortex hormones and thyroid hormones in susceptible pigs seems to be higher (Marple et al., 1972) (Marple & Nachreiner, 1976).

The interpretation of the hormone levels in itself is rather a problem. Several workers tend to give an increased importance to the role of catecholamines in triggering the PSE induction. The "fight or flight" reaction, mostly due to the catecholamines discharge, evokes in susceptible pigs often a fatal effect.

Furthermore physiological parameters such as respiration rate, heart rate, gas exchange parameters in the blood, enzym patterns have been studied in relation to this problem. Again the so-called post mortem feedback should provide here the ultimate answers.

Unshelm (1970) demonstrated that also within breeds, differences in exercise-tolerance exist which not always need to go along with muscularity. In blood samples metabolites and other parameters of muscle metabolism (p Co_2, p O_2, base excess, lactate, glucose, enzymes) indicate that the muscle system plays a key role.

The observed extreme acidosis and hyperthermia are the direct causes of the death of the animal. This suggests again that PSE is a symptom of a deviated muscle metabolism. The deviated muscle metabolism is also responsible for a higher death risk.

It goes without saying that the more the animal is selected for more muscle only, the normal balance between the different type of body tissues (bone tissue, fat tissue) and vital organs is endangered. But also within the muscle system itself it seems to be true. The relation between the anatomical-physiological pre-conditions and the required function of the different body systems (Unshelm, 1970) may be affected during this selection process.

Selection and tests

In order to reverse this development, without going back to a primitive kind of pig, we all were after practical tests which would provide us reliable criteria for use in breeding programmes. These tests should give enough information to use in genetic manipulation or measuring the extent of environmental influences.

Exercise tests with or without heat stress but also large shaking-devices were used (during the Wisconsin symposium in 1972) to measure the physiological stability. (Bünger et al., 1974; Steinhardt et al., 1974; Judge et al., 1973; Haase & Steinhauf, 1971; Kallweit & Haase, 1971).

The utilization of the laborious physiological parameters in this respect is hardly possible. This means also that it is difficult to get the right assessment of the genetic value with the help of this type of tests only.

Enzyme diagnostics give indications about the amount of leakage of the tissue bound enzymes into the blood after inducing stress. It renders good results in culling herds (Richter et al., 1976; Addis et al., 1976). For breeding programmes the limits cannot easily be defined because of the high range of values. The definition of the limits is the ultimate determining factor here. The fact that a single halothane application evokes in susceptible pigs the MHS syndrome must be considered as a breakthrough in this area of research.

Muscle biopsies analysed for glycolytic intermediates have the advantage that there exists a close relationship with the post mortem metabolic rate (Schmidt et al., 1971; Sybesma & Van der Wal, 1974). But this method requires surgical-like procedures. In acute Back Muscle Degeneration and Necrosis, a PSE-like problem, biopsies as diagnostic are very conclusive (Bickhardt et al., 1972).

The halothane test mechanism is in full agreement with the hypothesis of the adaptation theory of Selye. The adaptation reaction may be produced by different specific stimuli. In this case the halothane triggers the same (mal)adaptation reaction as for instance fright, heat or exercise. It is also suitable for use on young animals.

Work in our institute (Eikelenboom et al., 1976) and elsewhere (Christian, 1976) suggest a simple recessive inheritance of the MHS. Rasmussen & Christian (1976) demonstrated a coincidence with certain bloodgroups. Williams et al. (1976) on the contrary believe in genetical dominance of what they call Fulminant Malignant Hyperthermia.

In this conference more details will be given about the genetic value of several of the forementioned methods.

Handling

I mentioned the fact that fright often

acts as a trigger of the MHS syndrome. Translated in biochemical terms one might say that this underlines the theorie that catecholamines play an important aetiological role in PSE meat (Topel et al., 1974). This means that handling and managing, especially housing, must be kept in mind when besides genetic measures one has to reduce the chance for death losses and PSE occurence. Pigs are very clever animals, it is rather surprising to note how fast these animals adapt themselves to new environments (Van Putten, 1976). Therefore handling and managing with care must give them the opportunity to get accustomed to the new situation. In this field not enough work has been done.

Conclusion

Selection for muscle only induces the forementioned stress-prone pigs. When we conclude that the physiological imbalance of the pig which leads to the MHS and the PSS is a precondition for PSE meat there still needs to be ascertained whether the frequency of PSE meat in halothane negative pigs is acceptable from the production point of view. I hope I made it clear to you that in this sense, producing animals with an inbuilt disposition for PSE meat makes it understandable that PSE meat is listed in the category production diseases even if it is hardly pathological and the animal is already dead.

In my opinion the development of the research in the last twenty years from the post mortem to the ante mortem area gave us the tools to treat effectively the non-acceptable side effects of one of the most important production diseases in pigs. What is true for the imbalance in muscle tissue is hopefully also true for the imbalance for other tissues as there is the bone tissue.

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INVESTIGATIONS ABOUT THE FREQUENCY OF PSE AND DFD IN PORK

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Summary

Measurements of the pH_1 and ultimate pH value of loin, M.long.dorsi, and ham, M.semimembranosus, have been made in 3485 progeny-test pigs, which were slaughtered after a minimum and medium of stress and of 798 commercial pigs. The pH_1 value below 5.8 for PSE and the ultimate pH above 6.2 have been chosen as criteria of evaluation. The frequency of the incidence of PSE and DFD quality has been examined whether the results of a random sample can be accepted as generally valid for pigs, slaughtered under identical conditions.

These results show that the frequency of PSE and DFD meat depends on a genetic basis (progeny test pigs or commercial pigs) as well as on the stress before slaughter. The deviations are almost twice as high in pigs from the progeny-testing stations as in commercial pigs. The PSE changes are clearly predominant at a minimum of stress when pigs were slaughtered in the own slaughter-house of the progeny-testing station. However, at delivery to the municipal abattoirs the frequency of DFD can occur more often than PSE, especially in ham. Conclusions cannot be drawn from results of the ham muscle to the conditions of the loin and vice-versa. The analysis of variance shows that part of variance which reflects the day of slaughter, at the pH_1 value in loin and ham (4,0 - 11,6 %) is essentially lower as at the ultimate pH value (20,2 - 27,9 %). The taking of a random sample proved to be more certain in animals of known history and a minimum of stress than of animals with higher stress delivered to commercial abattoirs.

Introduction

Very different opinions exist about the frequency of the incidence of undesirable meat properties in pork. The percentages are reported to range between 5 % and 30% and are related predominantly to the PSE condition (Barton,1971; Bendall et al., 1966; Briskey,1963; Buchter & Zeuthen, 1971; Kempster & Cuthbertson,1975;Scheper, 1971,1972; Taylor,1966; Verdijk,1972). Data about DFD quality are available in a few cases (Bendall et al.,1966;Kempster & Cuthbertson,1975; Scheper,1972,1976). Besides that, there is uncertainty whether the reported data for specific populations can be accepted as generally valid or whether they have validity only for the investigated sample taken.

Materials and Methods

The investigations were carried out with fattened pigs of one hundred kilograms of weight from progeny testing stations (Herdbook), after a minimum of stress (0.2 km transportation on a lorry and slaughtered in the own slaughter-house = test I), and after a medium stress (30 km transportation and slaughtered in a municipal abattoir = test II), and with commercial pigs from the market, which were slaughtered in a municipal abattoir after 200 km transportation (test III). Electrical stunning (70 Volts) has been used in all tests.

The pH_1 value below 5.8 for PSE and the ultimate pH value above 6.2 have been chosen as criteria of evaluation, because these limits are correlated with essential changes in meat quality.

Results and discussion

A comparison of the 3 tests (table 1) showed that at the pH_1 value the parameters (mean value, standard deviation and distribution) in the loin are not identical to the data of the ham muscle. These results concur with the observation of Barton, 1971. The comparison of tests I and II of genetically comparable material (progeny test animals) points out that with a greater stress during transportation, the PSE frequency decreases,

Table 1: pH_1 and pH_{24} values in loin and ham muscles

Muscle		M. long. dorsi			M. semimembranosus		
Test		I	II	III	I	II	III
Number of animals		2354	1131	798	2354	1131	798
pH_1	$\bar{x}$	6,15	6,01	6,30	6,44	6,29	6,35
	s	0,45	0,30	0,35	0,45	0,42	0,33
	< 5,8 %	29,2	18,3	10,4	11,1	9,9	6,5
	5,8 - 6,2 %	22,5	53,0	25,1	17,5	28,0	24,3
	6,2 - 6,6 %	30,6	23,1	41,2	31,8	36,8	46,4
	> 6,6 %	17,7	5,6	23,3	39,6	25,3	22,8
pH_{24}	$\bar{x}$	5,59	5,82	5,71		5,81	5,78
	s	0,19	0,21	0,26		0,22	0,28
	< 5,8 %	88,1	39,2	70,9		43,0	58,7
	5,8 - 6,2 %	10,3	54,2	24,6		50,6	34,7
	6,2 - 6,6 %	1,4	5,8	3,6		5,4	5,3
	> 6,6 %	0,2	0,8	0,9		1,0	1,3

while the DFD part increases. Contrary to this result, the PSE part is essentially smaller in commercial pigs than in pigs from progeny-test stations, without a corresponding increase in the DFD part. With these results it may be possible to draw conclusions about a sample taken randomly from the ham muscle concerning PSE as well as DFD. Contrary to this there is no evidence concerning the loin, because in this case the percentages differ considerably with 10 % (III) and 29 % (I). This becomes even more distinct when both, PSE and DFD, are taken into consideration; the following per cents were revealed for the loin muscle 30,8 % (I), 24,9 % (II) and 14,9 % (III) and in the ham muscle 11,1 % (I only PSE), 16,3 % (II) and 13,1 % (III). To some extent the mean values at larger test series can be transferred to a shorter period of time when compared on a monthly basis. The results of the same months in a three year period of test I were combined, because similar tendencies were observed. During the months from April to June the highest pH_1 mean values exist and the lowest deviations occur in line with PSE, and during the months of December until February the most frequent PSE changes were determined at lower pH_1 values. If the pigs are under the same stress before slaughtering as in test I the mean values at approximately the same standard deviations allow us to draw conclusions about the PSE-part. In this case, pH_1 mean values lie between 6,0 and 6,1, the parts of PSE is changing above 30 % and at pH_1 mean values above 6,2 between 17 % and 23 %. These findings are generally not transferable under other conditions, e.g. test II, with its lower pH_1 level.

The existing tendency of a seasonally caused influence in test I is not given in tests II and III. The percentages in the months of September (PSE and DFD: II = 35,6 %, I = 35,0 %) and in November (II = 38,5 %, I = 31,8 %) are almost comparable, but not in the months of December (II = 14,6 %, I = 40,7 %), and January (II = 14 %, I = 38,5 %). In this connection it is important that during the strong decrease of the PSE part in the months of December and January (test II) the DFD part does not correspondingly increase. It is remarkable that the monthly differences in commercial pigs are considerably smaller than in those from the progeny testing stations.

The conditions of the ham muscle do not apply to those of loin and vice-versa. There is an indication that at a minimum of stress (I) the changes in PSE in the loin occur twice or three times as often as in ham. By applying a higher stress

(II and III) the quota of deviation(PSE and DFD) in both parts becomes smaller with a stronger increase of the DFD quota in the ham muscle. Under these circumstances the DFD part may predominate (loin: test II Dec., Jan.; ham: test II Oct., Dec., Jan.; test III Sept.,Oct., Febr.).

Since no uniform tendency occurs during the individual day of slaughter in a month it has been examined how the day of slaughter influences the initial pH value (pH_1), and the ultimate pH value (pH_{24}).

Analysis of variance shows that the part of variance, which reflects the day of slaughter, at the pH_1 value in loin and in ham, is essentially lower as at the ultimate pH value (table 2).

Table 2: Analysis of variance,influence of the days of slaughter

	Test	Days n	Part of total variance Loin %	Ham %
pH_1	I	145	8,2	10,6
	II	21	11,3	8,8
	III	6	4,0	5,4
pH_{24}	I	145	21,9	
	II	21	27,9	21,4
	III	6	20,2	21,3

These per cents of variance are especially at the pH_{24} value considerably lower than those found by Charpentier et al.,1971. One should note, that no considerable differences exist between loin and ham muscle. With regard to pH_1 value, the pigs from the progeny-testing station (I, II), react more strongly than commercial pigs (III). Bendall et al., 1966,reported also these differences. In the reaction on the ultimate pH value a far-reaching conformity exists in all tests. This result confirms former data indicating that the effect of environmental factors on the PSE condition (pH_1) is lower than the genetic influence which is approximately 20 % of the total variance (Scheper, 1972).

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PALE, SOFT, EXUDATIVE (PSE) MEAT, STRESS SUSCEPTIBILITY & MHS IN PIGS - ENDOCRINOLOGICAL & GENERAL PHYSIOLOGICAL ASPECTS

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Summary

The study of stress sensitivity in pigs has been aided in recent years by the finding that certain drugs eg. halothane or suxamethonium, can trigger a metabolic reaction - so called Malignant Hyperthermia (MHS) which shares many features of the normal reaction of stress sensitive pigs to stunning and slaughter. Moreover these observations suggested the use of halothane sensitivity tests for the identification of pigs likely to suffer from the Porcine Stress Syndrome or produce meat of inferior quality. It is important, therefore, to identify the physiological basis of MHS and of animals' reactions to stunning and slaughter.

Recent discoveries in our laboratory have shown that MHS is characterised by metabolic changes indicative of severe muscle stimulation. There is a fall in the Free Thyroxine Index (FTI) in the serum which occurs simultaneously with, and may be the result of a substantial rise in plasma catecholamines. Curare, in dangerously high doses, or pancuronium can create a neuromuscular blockade which may modify the response and even protect some pigs from developing a fatal response to the triggering agent. Infusion of pigs with large doses of the α-adrenergic blocking drug, phentolamine, will prevent a response; β-adrenergic blockade will not. All these features can be observed in the reaction of stress sensitive pigs at slaughter and α-, but not β-, adrenergic blockade will promote the production of meat of acceptable quality in such pigs.

These results support the use of MHS as a model for investigations of sensitivity to stress and the production of pale, soft and exudative (PSE) meat in pigs. Their relation to the hormonal control of metabolism, growth and body composition in pigs is discussed.

Introduction

Rigor mortis develops 4-6 hours after pigs die. It is characterised by the rigidity of the musculature, which give the condition its name, the accumulation of lactate, hydrogen ions and inorganic phosphates in and the disappearance from muscle of glycogen and energy rich phosphates. This process varies from animal to animal only in the extent of the changes and the rate at which they continue. Individuals of a species also vary in these respects and in pigs the variation is responsible for the spectrum of meat quality ranging from PSE (pale, soft and exudative) to DFD (dark, firm and dry). These conditions are produced by well understood processes involving the denaturation of the soluble and structural proteins of muscle which in turn affects its water-binding and light-scattering properties. It is the rapidity of glycolysis and specifically the mechanism responsible for triggering it which probably provide the common link between the three syndrones presently under consideration.

It has been our experience that the metabolic and physiological changes which characterise PSE meat production, stress susceptibility or MHS are essentially similar to those found in normal individuals after any severe muscle stimulation such as exhaustive exercise (Pernow & Saltin, 1971). The problems arise only when the stimulation is so severe that the pig is incapable of controlling the severe catabolic state and entering a recovery phase or, as can be the case with PSE meat, the animal is slaughtered during the developing reaction or at its height. One might imagine that the trauma of the slaughter process would have considerable impact on the metabolism of any animal, but PSE meat can be found in the carcasses of animals which die without encountering any apparent stress or in conditions which favour the production of excellent meat in the overwhelming majority of carcasses.

Training or acclimatisation to the experimental conditions and personnel is often necessary before experimental animals are subjected to metabolic investigations. This is to ensure that the heat production of animals is basal at the time of measurement (Blaxter, 1962). There is no doubt that some animals destined for slaughter might benefit in terms of the ultimate quality of their meat if they were 'trained' to accept the pre-slaughter handling. This however cannot provide a complete explanation for the metabolic responsiveness of animals as researches on neuromuscular stimulation and glycolysis have shown. By the use of the neuromuscular blocking drug tubocurarine in anaesthetised pigs of the Large White type the rate of glycolysis and pH fall in

muscle post mortem can be retarded several fold (McLoughlin, 1963; Bendall, 1966). Pigs such as Pietrain and Poland China which are markedly sensitive to stressful stimuli are but little affected by curarisation in terms of glycolysis in their muscles post mortem and the poor quality of their meat is almost unchanged (Lister, 1970; Sair et al 1970). This suggests that the muscle of pigs like Pietrains or Poland Chinas is constantly or more readily stimulated than that of Large Whites and conventional procedures for neuromuscular blockade are less effective.

Swatland & Cassens (1972) reported that the motor end-plate was more extensive in the muscles of stress susceptible than resistant pigs. Thus the possibility exists that the doses of curare used in the latter investigations were inadequate for complete blockade in stress susceptible pigs. Curare, in very large doses, is not tolerated by animals because of its hypotensive effects. Pancuronium, which is also a non-depolarising blocking drug, can, however, be given without undue hazard in doses which will significantly retard glycolysis. This again does not provide the complete solution, for an even greater retardation of glycolysis can be induced by the intravenous injection of magnesium sulphate (Lister, 1970; Lister & Ratcliff, 1971, Sair et al., 1970). Magnesium sulphate has neuromuscular blocking properties similar to curare and specifically inhibits myofibrillar ATPase activity. The relevance of these effects, though likely, is not completely clear. It may well be that Mg^{2+} ions have a more important role to play in the modification of Ca^{2+} flux in tissues and consequent influence on excitation contraction coupling (Lehninger, 1970; Cheah & Cheah, 1976).

Malignant Hyperthermia (MHS)

Body temperature provides an important indicator of the metabolic state. Even at rest Pietrain pigs frequently show a higher rectal temperature than Large Whites. Moreover their body temperature is easily raised further by any stressful stimulus, a symptom of the so-called Porcine Stress Syndrome (Topel et al., 1968) and one which Sybesma & Eikelenboom (1969) considered to be an important contributory factor in the death of affected animals. Interest in the control of temperature has grown in recent years as a consequence of the widespread investigation of the syndrome known as Malignant Hyperthermia (MHS) which affects pigs and a number of other mammalian species including the human being (Lucke, 1976) and a consideration of this most important investigative tool will provide the basis for the remainder of this paper.

MHS has been triggered experimentally and accidentally in pigs and people by a wide variety of agents the most common of which are suxamethonium and halothane (Britt & Kalow, 1970) but an MHS like syndrome can be induced in animals following the stress of the chase and capture (Harthoorn et al., 1974) and even by the mental stress of being party to but not the subject of venepuncture (Lister et al., 1975).

MHS induced by suxamethonium is characteristically more violent and of quicker (seconds to minutes) onset than that resulting from exposure to halothane (up to an hour or more on occasion) (van den Hende et al., 1976). Pigs may differ in the time they take to react and the rate and extent of the subsequent reaction but the metabolic and physiological changes are essentially similar (Lucke et al., 1976). Neither are they affected by the triggering agent employed.

The onset of the reaction after suxamethonium is frequently accompanied by violent muscle fasciculations often merging into rigidity. The latter may not be seen after halothane until terminally and the reaction generally is more insidious. An early sign of MHS is a change in the end-tidal gas concentration of carbon dioxide which accompanies the increase in blood PCO_2 and fall in pH. Increases in plasma lactate and inorganic phosphate are universal findings (Berman et al., 1970; Lucke et al., 1976; Gronert & Theye, 1976). There are changes in electrolyte concentrations in blood, some of which reflect haemoconcentration, eg. Ca^{2+}. Mg^{2+} and K^{+} increase to a greater extent than haemoconcentration would indicate; Na^{+} and Cl^{-} less. Altered membrane permeability is indicated also by the raised concentration of Creatine Kinase and Lactate Dehydrogenase.

Although the name of the syndrome implies that significant increases develop in body temperature they are only commonly but not universally found (Lucke et al., 1976). Neither, despite many suggestions to the contrary, is there good evidence of cardiomyopathy. Indeed in our experiments with Pietrain pigs cardiac output was readily stimulated twofold and it was only terminally that arterial pressure fell and gross disturbance of the e.c.g. were apparent (Lucke et al, 1976).

The reported changes in the cardiovascular system are associated with massive increases in plasma catecholamines (Lister et al., 1974; Gronert & Theye, 1976; van den Hende et al., 1976) of which noradrenaline predominates. There is frequently, though not always, a concomitant marked

hyperglycaemia. Plasma free fatty acids decline presumably as a consequence of the acidosis (Boyd et al., 1974).

Source of Heat Production

There is now little doubt that the primary site of heat production in MHS is skeletal muscle (Britt, 1972; Relton et al., 1973) although there is undoubtedly some contribution from the liver (Berman et al., 1970) despite indications of its impaired function (Evans et al., 1975). Whether the heat is derived primarily from aerobic or from anaerobic muscle metabolism is not so clear. Berman et al. (1970) and Berman & Kench (1973) reported that up to 50% of the heat produced in halothane induced MHS was anaerobically derived whereas Gatz (1973) argued on theoretical grounds that thermogenesis in MHS was predominantly aerobic in origin. Our own estimates (Hall et al., 1976) based on suxamethonium and halothane induced MHS suggest that aerobic muscle metabolism is responsible for almost all the heat produced in the initial stages and about 50% terminally. The method of stimulating muscle appears to have little effect on these proportions. In our pigs a difference of 2.6°C was seen terminally between the observed and temperatures calculated to result from the oxidation of glucose. The lactate accumulating in the muscle can be shown to be that which would be produced by anaerobiosis to account for the extra heat production (Hall et al., 1976).

Treatment

Although the suggested treatments and preventive measures for MHS, stress susceptibility and the avoidance of PSE meat production are legion, most are designed to alleviate symptoms and not to deal with causal mechanisms. This is understandable, when no clear cause has been established, but two approaches have provided rewarding results. The provision of an environment in which stressful stimuli are minimal, tranquillisation or premedication with drugs have all been shown to reduce the incidence of adverse reactions (Devloo et al., 1971; McLoughlin & Heffron, 1975; Britt et al., 1975) but the procedures designed specifically to prevent neuro muscular stimulation have been most consistently effective. Prevention is always preferable to treatment of the reaction for once initiated the metabolic events are difficult to control and eventually a stage is reached when they are virtually impossible to reverse (Hall et al., 1975). The acidosis and irregularities of the e.c.g., on the other hand, are relatively easy to correct by bicarbonate infusion (Britt & Kalow, 1970) and β-blockade (Hall et al., 1975) but the primary reaction may continue unchecked.

In recent work (Hall et al., 1976) we have attempted to relate the early observations on neuromuscular blockade with non depolarising relaxants in stress susceptible and stress resistant pigs to MHS. When tubocurarine was given to Pietrain pigs, the usual fatal response to suxamethonium was prevented although it provided no protection against halothane induced MHS. Pancuronium, given in greater doses provided some protection even against halothane for half of the pigs studied reacted and died only when the neuromuscular block was reversed by neostigmine. Pancuronium could not reverse an established reaction. These results suggest that the triggering of MHS by suxamethonium required the depolarisation of the motor end-plate of muscle as might be expected to occur in a stressed, conscious subject. The action of halothane cannot easily be explained in this way, but protection may relate to the almost totally inactive state and Ca^{2+} unresponsiveness of muscle suffering a high degree of neuromuscular blockade. This would support the views of Berman (1973) and Mogensen et al. (1974) who proposed that, in human cases of MHS, previous muscle activity could modify its sensitivity to triggering agents.

The local anaesthetics procaine or procainamide have been used with apparent success in the treatment of MHS (Beldavs et al., 1971; Harrison, 1971; Brebner & Josephowicz, 1974) but Hall et al. (1972) were unable to confirm the efficacy of procaine. Moreover Hall & Lister (1974) considered that it was not possible to obtain clinically the tissue levels of procaine which were indicated for muscle relaxation by in vitro experiments.

A further approach was suggested by Lister et al's. (1974) observation, subsequently confirmed in other experiments (Gronert & Theye, 1976, van den Hende et al. 1976), that there was a massive rise in circulating catecholamines, predominantly noradrenaline, which was strongly correlated with the rise in plasma lactate (Lucke et al. 1976) and a fall in the Free Thyroxine Index (Lister et al. 1974). α adrenergic blockade with large doses of phentolamine successfully prevented MHS after the subsequent administration of suxamethonium although the initial muscle response to the drug still occurred. Neither could an established response be reversed. β-blockade with propranolol neither prevented the initial reaction to suxamethonium nor its persistence and the development of MHS.

The beneficial effects of α adrenergic blockade prior to slaughter on the ultimate quality of meat have now been confirmed. Propranolol administration also led to a small improvement in meat quality, but only

that which could be accounted for by its local anaesthetic and not by its β-blocking properties (Lister, 1974).

The most effective agent so far identified for both the prevention of MHS and treatment of an established reaction induced by halothane is the phenytoin derivative, dantrolene. Bianchi (1973) suggested that diphenylhydantion, which acts both pre- and post-synaptically to reduce the excitability of muscle fibres and alter Ca^{2+} efflux from the cell, might be of value in the treatment of MHS. Harrison (1975) using dantrolene, which structurally resembles diphenylhydantoin, successfully treated a number of episodes of MHS. Gronert et al. (1976) and Hall et al. (1976 - unpublished results) have confirmed these findings. Investigations on the effectiveness of dantrolene on changes in muscle post mortem and meat quality have not yet been completed.

Mechanisms

It has been generally recognised that like the source of heat production the final event in the chain of physiological reactions which characterise MHS was to be found in muscle. 'Uncoupling' in mitochondria was offered as one explanation (Eikelenboom & van den Bergh, 1971) but this has been shown not to be the case (Brooks & Cassens, 1973; Cheah, 1974; Campion et al., 1975). A specific uncoupling effect of halothane now seems to be unlikely on theoretical grounds (Williams, 1973).

A defect in the ability of the sarcoplasmic reticulum (SR) to handle Ca^{2+} has also been postulated but unpublished observations from our own laboratory (Ketteridge,1970) were unable to demonstrate differences in Ca^{2+} binding ability of the SR or myofibrillar ATPase activity between Pietrain and Large White pigs and similar findings have been described for stress susceptible and resistant pigs by others (Greaser et al., 1969). Moulds & Denborough (1974) considered amongst other criteria that a raised concentration of Ca^{2+} in the myoplasm might result from the increased release of Ca^{2+} from the sarcolemma and the SR. Hall et al. (1973) and Britt et al. (1965) have described specific effects of halothane on mitochondrial respiration and Ca^{2+} accumulation. Direct evidence for enhanced Ca^{2+} efflux from mitochondria prepared from Pietrain muscle in response to anaerobiosis and halothane and its inhibition by Mg^{2+} has recently been provided in our laboratory (Cheah & Cheah, 1976). They also postulate that since there is no difference in the Ca^{2+} accumulating ability of SR between Pietrain and Large White or stress susceptible and stress resistant pigs any excess Ca^{2+} released by anaerobiosis in Pietrains or similar pigs will not be taken up by SR and will activate myofibrillar ATPase and phosphorylase kinase. As acid production increases, the Ca^{2+} accumulating ability of SR is depressed and a vicious cycle of Ca^{2+} release and contraction is initiated. Halothane can thus act in MHS by triggering mitochondrial Ca^{2+} efflux and by inhibiting Ca^{2+} intake by SR (Britt et al., 1975). These observations provide evidence for possible sites of action for the effects of Mg^{2+} on the control of post mortem glycolysis (Sair et al., 1970; Lister & Ratcliff, 1971) and in MHS (Lister, 1973; Lucke, 1976). They also suggest a site of action for dantrolene other than the SR.

Comment

Wingard (1974) suggested that malignant hyperthermia in human beings was another manifestation of a generalised stress syndrome. It has, of course, been known for many years that those breeds of pig which commonly develop MHS eg. Pietrain, Poland China and Landrace also react to various stressful stimuli such as physical exercise or raised environmental temperature, and frequently develop acidoses, muscle tremor and fever and many die (Topel et al., 1968). The Pietrain is one of if not the most sensitive of all the modern breeds of pig and many develop MHS with minimal provocation. Pietrains suffer a high incidence of unexplained deaths such as Wingard (1974) noted in his human families.

Our findings on the involvement of catecholamines and especially their α effects in both MHS and the production of PSE meat suggest an important role for these hormones in the pathogenesis of these syndromes.

We envisage that the part played by catecholamines in the development of MHS can be explained as follows: The initial muscle stimulation, produced by the triggering agent, is grossly exaggerated in sensitive pigs and causes a marked metabolic acidosis and a hypercarbia. These early metabolic events do not resolve spontaneously for reasons not yet apparent. On the contrary muscle metabolism is stimulated further under the influence of catecholamines and the original metabolic changes are exacerbated. It is significant that, for protection against suxamethonium, sensitive pigs require massive α adrenergic blockade. Once the reaction is established, it is almost impossible to create the necessary degree of α block in the fact of the observed concentrations of agonist (~ 50 μ g/l plasma).

Thus a progressive and malignant reaction is established whereby metabolic changes

induce the release of catecholamines which, whilst providing for the mobilisation of energy substrate, also further stimulate the metabolic events for which the energy was initially required.

The involvement of catecholamines also suggests a common link between various, apparently unrelated, predisposing factors such as pre operative excitement and apprehension (Mogensen et al., 1974) excessive muscular activity and trauma (Berman, 1973) and possibly, the use of anti cholinergic drugs (Kalow, 1972). When a rise in body temperature occurs during anaesthesia it might, therefore, be due to the stress of induction and not necessarily to some of the drugs implicated as triggering agents.

Susceptibility to MHS in pigs is found in those breeds which develop lean carcasses. This is associated with, and may be the result of, an exaggerated lipolytic response to catecholamines (Lister, 1976). It is of note that several of the reported human cases have also been strikingly mesomorphic. Thus it might be that a genetically fixed characteristic of some breeds of pig may appear less frequently in the human population but with the same potentially fatal outcome during anaesthesia or acute stress.

Cheah & Cheah's (1976) idenfitication of the altered Ca^{2+} flux from mitochondria of stress susceptible animals after exposure to halothane or during anaerobiosis provides direct evidence for a link between the postulated biochemical changes and excitation contraction coupling and heat production in muscle. It is, however, difficult to understand why cardiac muscle continues to function adequately in the presence of halothane whilst skeletal muscle is so dramatically stimulated.

If, however, mitochondrial Ca^{2+} provides an ultimate trigger for excitation contraction coupling in muscle (Lehninger, 1970) it is possible that mitochondrial Ca^{2+} is also the stimulus for excitation-secretion coupling and hence the unusual hormone picture to be seen in animals which develop MHS.

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BLOOD LEVELS OF INSULIN, TRIIODOTHYRONINE AND THYROXINE IN GERMAN LANDRACE PIGS AND THEIR RELATIONSHIPS TO MEAT QUALITY (PSE)[1]

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Summary

Hormone assays were performed in blood of female pigs (German Landrace). The rapid pH decrease 45min. post mortem to 5,5 and Göfo values of 52 in the m.long.dorsi indicate that PSE-meat frequently occurs in this breed. The mean values of insulin, T3 and T4 were 27 µU/ml, 0,97 ng/ml and 4,25 µg/100ml, respectively. No correlations were found between levels of insulin and meat quality. However, significant correlation coefficients (0.36 and 0.28, resp.) were observed between T3, resp. T4 and serum lactate. The correlations between the two hormones and pH of m.long.dorsi were -0.14 per each. The results point to a glycolysis promoting effect of thyroid hormones in pigs.

Introduction

Earlier investigations of Bickhardt (1972) and of many other authors show that in PSE-susceptible pigs the glycolytic system is considerably affected. Possibly insulin takes part in the process leading to poor meat quality. It is well known to increase the intracellular glycogen depot - the prior condition for an augmented post mortem glycolysis. With regard to glycogen metabolism Newsholme & Start assume an insulin-adrenaline interaction which is of importance especially during stress.

Some relevance for the occurence of PSE-meat may belong to the thyroid hormones. Recently Pfeifle (1976) observed that T3 and T4 stimulate the ß-receptor binding of catecholamines. Several investigations emphasize the meat quality deteriorating effects of adrenaline (Seifart, 1962; Haid et al., 1973; Rogdakis & Haid, 1974) whereas corticosteroids seem to have no influence in the development of PSE-meat (Steinhauf et al., 1969; Haid et al., 1973; Kraeling et al., 1975; Rogdakis et al., 1975).

Materials and methods

Female German Landrace pigs were kept at the Mastprüfungsanstalt Forchheim (ad lib. feeding during fattening period between 30 and 100kg body weight). Before killing the pigs were withheld from feeding about 20hrs. Blood sampling was performed immediately after immobilization by CO_2-inhalation. Deproteinized blood served for glucose (GOD-Perid method) and lactate determination (test-combination Boehringer, Mannheim). Serum was used for insulin, triiodothyronine (T3) and thyroxine (T4) evaluation.

The hormones were determined radioimmunologically by kits of the Behring-Werke (RIA-gnost Insulin) and Henning-Berlin (T3-RIA). Insulin and T3 assays were made without previous extraction. Separation of free from bound antigen has been achieved by Amberlite and charcoal for insulin and T3 assays, resp. After alcohol extraction the total thyroxine was determined according to the principle of protein binding analysis. pH and Göfo values were measured as described previously (Haid et al., 1973).

Results and discussion

1. Meat quality

The values shown in table 1 indicate a frequent occurence of PSE-meat in the examined population.

Table 1. Average pH and Göfo of m.long.dorsi and serum lactate concentration.

Item	Mean ± SEM	No. of animals
pH (45min.p.m.)	5.5 ± 0.02	182
Göfo (24hrs.p.m.)	52.0 ± 0.62	177
Lactate (mg/100ml)	73.4 ± 3.43	74

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2. Blood levels of insulin, triiodothyronine and thyroxine

No comparable data could be found for insulin in the available literature. The values of T3 and T4 are in good agreement with the data published recently by Marple et al. (1975). The low blood glucose levels were possibly induced by the relative long fasting before slauthering.

Table 2. Insulin, T_3, T_4 and glucose levels in blood.

Item	Mean ± SEM	No. of animals
Insulin (µU/ml)	27.0 ± 1.25	166
T_3 (ng/ml)	0.97 ± 0.05	79
T_4 (µg/100ml)	4.26 ± 0.15	155
Glucose (mmol/l)	3.39 ± 0.10	166
(mg/100ml)	61.1 ± 1.82	

The correlation coefficients for the hormones and blood glucose are presented in table 3. There was a positive correlation between insulin and blood glucose and a negative one between thyroid hormones and blood glucose.

Table 3. Correlation between hormone levels and blood glucose concentration.

Variables	Correlation Coefficients	No. of animals
T_3/Insulin	$-.32^{+}$	52
T_4/Insulin	.13	147
T_4/T_3	-.01	56
Glucose/Insulin	.18	150
Glucose/T_3	-.19	53
Glucose/T_4	$-.29^{++}$	139

3. Relation between hormone levels and criteria of meat quality

No significant relation was found between serum insulin and the criteria of meat quality. Significant positive correlations were observed between thyroid hormones and blood lactate. The correlations between T3 and T4, resp. and the pH value of m.long.dorsi were negative but not significant. The results indicate that there is probably a glycolysis promoting effect of thyroid hormones in swine.

Table 4. Correlation coefficients of hormone values and criteria of meat quality (plus serum lactate).

	Criteria of meat quality and serum lactate		
	pH	Göfo	Lactate
Insulin	-.03(165)	-.04(150)	-.07 (66)
T_3	-.14 (78)	.10 (54)	$.36^{+}$(24)
T_4	-.14(154)	-.04(150)	$.28^{+}$(58)

Our findings do not agree with the hypothesis of Ludvigsen (1968) that a low thyroid activity is associated with or even a cause of PSE-meat. In contradiction to his supposition are also the reports of Eikelenboom & Wiess (1972), who found higher T4 levels in PSE-susceptible pigs (Piétrains) and of Haid & Ensinger (1975) who observed also a higher T4 blood concentration in Piétrains than in the German Landrace and intermediate values in F_1-pigs. Marple et al. (1975) demonstrated a more rapid post mortem glycolysis in T4-fed pigs than in untreated or thyroidectomized animals. The inhibition of catecholamine-induced glycogenolysis in hypothyroid rats or patients (Fregly et al.,1975; Rosenqvist, 1972) and the potentiation of the hyperlactacidemic effects of adrenaline by T4 in rabbits (Svedmyr, 1965) support the assumption of a glycolysis stimulating effect of thyroid hormones. Obviously the hormones can modify the effects of catecholamines. T3 and T4 may alter the response of adrenergic end-organs at several levels: (1) by altering

the number or sensitivity of catecholamine receptors, (2) by altering adenyl cyclase activity, (3) by altering intracellular processes influenced by catecholamines (Spaulding & Noth, 1975). Recent work of Pfeifle (1976) favours the first possibility. She found an increased binding of catecholamines to ß-receptors in the presence of thyroid hormones. From this point of view our findings do well agree with the results of Eikelenboom and Marple establishing further evidence for a glycolysis promoting effect of thyroid hormones in pigs.

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NEURONAL CONTROL OF MUSCLE PROPERTIES[1]

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Summary

Various characteristics of motor innervation of muscle were studied in relationship to the properties and spatial distribution of muscle fibers. Most skeletal muscles of the pig have a unique grouped arrangement with 3-70 type I fibers occurring in discrete clusters surrounded by type II fibers. The deep red portion of the semitendinosus muscle exhibits typical type grouping and the superficial portion has type II predominance. Experimentation revealed that the motor end plates in the deep red portion were larger than those in the superficial white portion, and innervation ratios were near 1.00 in both portions of the muscle. Type grouping in normal skeletal muscle of the pig is, therefore, not the result of multi-fiber innervation by subterminal axons, but may be the manifestation of a unique motor unit topography. Nerve crush of the upper sciatic nerve followed by subsequent reinnervation produced fiber type conversion which resulted in a fiber type grouping pattern dissimilar to the normal grouped arrangement. Significantly ($P < .01$) elevated terminal innervation ratios were found in reinnervated muscle as a result of extensive branching of the subterminal axons, but the percentages of type I & type II muscle fibers were unchanged. It was concluded that the neurone exerts a strong influence on the muscle fibers it innervates; this suggests a potential for controlling muscle properties in meat producing animals.

Introduction

Muscle, for use as a food, exhibits an enormous variation in chemical and physical properties and organoleptic characteristics. Meat scientists have worked at developing evaluation methods, ranging from live animal and carcass classification systems to characterization of muscle proteins, in an attempt to describe adequately the variation known to exist. Others have attacked the problem by investigating the influences of genetics and environment on muscle properties with the hope that variation in muscle properties could be minimized by management techniques. Special problems, such as PSS and PSE, have caused meat researchers to delve deeper into basic muscle function and influence of the endocrine system in order to learn about the mechanism of the problem. Now, with a growing awareness of potential world food problems and with the more common use of plant proteins in meat products, there is an increased need for understanding and control of the variation so that the meat can be utilized to greatest advantage.

Muscle is composed of muscle fibers; during the early 1960's meat scientists recognized that the population of fibers was not homogeneous but rather composed of at least two distinctly different types known broadly as red and white. The proportion of fiber types in a muscle determines the gross properties of the muscle such as color, as well as specific basic properties such as enzyme profiles. Given muscles, because of their function in the body, always fit into a given pattern; for example postural muscles are usually red while others which are called on for short periods of hard work are white. Variation of fiber type composition within a given muscle does occur and offers a possible explanation for PSE, differences in muscle growth, and variations in meat curing.

The neuronal control of muscle properties has received little attention from meat scientists. At present we believe it offers the most likely opportunity for progress. This manuscript will present a synopsis of pertinent literature and then explain the efforts being conducted at the University of Wisconsin to establish the extent and mechanism of neuronal control of muscle properties in the pig. The work is being conducted as a background to assessing application in the meat production industry.

Motor neurone cell bodies are located in the ventral gray horn of the spinal cord. An axon extends from a cell body, via a peripheral nerve, to a muscle. Branching of the axon occurs before and after the nerve enters the muscle and the branches from a given axon are distributed about the innervation zone of a muscle by intramuscular nerves. When a branch of the axon leaves

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the intramuscular nerve it is a terminal axon; contact is made with the muscle fiber by an arborization of the terminal axon known as the motor end plate. The group of fibers (which may number from a few to several hundred) innervated by the branching of an axon from one motor neurone cell body is a motor unit. The fibers of a motor unit are dispersed about the muscle, contract simultaneously and all have the same histochemical, physiological and biochemical properties (Edström & Kugelberg, 1968; Burke & Tsairis, 1974).

The cross-innervation experiments of Buller et al. (1960) established that the speed of contraction of muscle is under strong neural control. They transplanted nerves from fast and slow muscles so that fast neurones innervated slow muscles and slow neurones innervated fast muscles. Results showed clearly that the slow muscle gained a faster contraction time and the contraction time of the fast muscles decreased. Subsequent work revealed that the neuronal influence, in fact, converted properties of the myofibrillar protein, myosin (Samaha et al., 1970; Sreter et al., 1974; Weeds et al., 1974).

Only limited application has been made of knowledge about the neuronal control of gene expression in muscle. In meat producing animals innervation has been suggested as a control agent in muscle development (Swatland & Cassens, 1973, 1974) and as a factor in double muscling of cattle (Swatland, 1973).

Normal mammalian skeletal muscle has a random distribution of fiber types. Grouping of fiber types occurs in certain neuromuscular disorders (Engel, 1965; Morris, 1969) and as a result of reinnervation Karpati & Engel, 1968). Type grouping results from reinnervation of denervated muscle fibers by collaterals from persisting healthy axons or regenerating axons (Engel, 1965; Karpati & Engel, 1968; Morris, 1969).

The skeletal muscle of pig has various degrees of type grouping (Moody & Cassens, 1968). One or several clusters of type I fibers are found in a fasciculus and are surrounded by type II fibers.

The highly patterned distribution of fiber types in pig muscle makes it an ideal model for study of neuronal control of muscle properties in a meat producing animal. Specifically, the significance of motor end plate size, the possibility of collateral sprouting as a factor in type grouping and the influence of reinnervation on fiber type grouping were all studied in pig muscle with a view to potential application in meat production.

Materials and Methods

Motor end plate diameter was measured in the superficial (white) and deep (red) portions of the semitendinosus muscle of 12 castrated male pigs of Hampshire or Chester White breeds and muscle fiber size was measured on 5 of the 12 pigs. Fourteen castrated male pigs (mean age = 219 days) were used to determine if collateral sprouting of axons was responsible for the type grouping in normal muscle of the pig. Twenty-seven castrated male Hampshire and Poland China pigs between 6 and 8 weeks of age (12.3 kg average body weight) were used to study denervation and reinnervation (see Beermann et al., 1976 for details). The upper sciatic nerve was exposed by incision and blunt dissection between the superficial gluteus and biceps femoris muscles in 18 of the pigs. The nerve was crushed for 15 sec. in the right leg by full closure of an eight-inch hemostatic forceps which had the tips covered with tygon tubing. A second crush was administered 5 mm distal to the first crush in 6 animals and 5 animals were sham operated by exposing but not crushing the nerve. Four animals which received a nerve crush were sacrificed at one week post nerve crush and the remaining animals were sampled at 10, 15, 20, 25, 27 and 31 weeks following nerve crush.

Appropriate muscle samples were removed from animals under surgical anesthesia, restrained at resting length and frozen in liquid nitrogen. Longitudinal thick sections (70-100μ) were cut in a cryostat and stained for intramuscular innervation by a modified acetylcholinesterase-silver nitrate method (Beermann & Cassens, 1976). Thin sections (10μ) were cut and reacted for ATPase after acid or alkaline preincubation (Guth & Samaha, 1970), phosphorylase (Pearse, 1972) and NADH-tetrazolium reductase (Engel & Brooke, 1966). Type I fibers had low alkaline ATPase and phosphorylase activity, and high acid ATPase and NADH-tetrazolium reductase activity. Type II fiber had the opposite characteristics. Type II fibers stained by the alkaline ATPase procedure had subclasses of dark and intermediate staining fibers but both were classified as type II. Functional (FTIR) and absolute (ATIR) terminal innervation ratios were determined according to Coërs et al. (1973).

Results and Discussion

Motor end plates were larger in the deep red portion (41.1 ± 4.6μ) of the semitendinosus than they were in the superficial white portion (35.7 ± 2.7μ); this difference was significant ($P < .01$), but the size of the muscle fibers was not different between the two portions of the muscle. A generally accepted conclusion from the literature is that larger muscle fibers are innervated by larger motor end plates (Ip, 1975; Nystrom, 1968). Kuno et al. (1971) have shown from direct experimentation that the amount of

transmitter released following nerve stimulation is related to size of the nerve endings. Dias (1974) noted, with one exception, that larger motor end plates were found in slow (red) muscle and suggested that the larger motor end plates in red muscle may be related to their continuous slow activity pattern. In pig muscle we found larger motor end plates in red muscle compared to white, but in the two portions of the semitendinosus a difference did not exist in fiber size. One conclusion is, as Dias (1974) has suggested, that more control is exerted over the red muscle by the nervous system because there are larger motor end plates which probably deliver more of a substance (trophic factor?) to the fiber. An alternative viewpoint is that less neural influence is exerted over white fibers. This point may be related to the concept of fiber type differentiation and growth - both of which are important to animal production. The white fibers may be white because they have less neural control and have undergone less differentiation than red fibers. Swatland and Cassens (1973) put forth the idea that two populations of myofibers were identifiable during development of pig muscle. The primary type is found first and generally lies centrally within a fasciculus; the secondary type appears later and lies peripherally in the fasciculus. They then speculated that the central myofibers are innervated by older axons and develop an oxidative type of metabolism compared to the peripherally located, later innervated fibers which develop anaerobic properties.

From our present data the area of contact of the motor end plate with the muscle fiber should be considered as playing some role in control of muscle properties. The reader must be fully aware that current extensive effort is being made to segregate the contributions of chemical transfer and stimulation pattern as two separate factors which influence properties of a muscle fiber (see Guth, 1975 for a discussion of this issue).

The occurrence of type grouping in muscle is associated with chronic peripheral nerve disorders (Coërs & Woolf, 1959; Morris, 1969) or reinnervation (Karpati & Engel, 1968) and is thought to be the result of reinnervation of denervated fibers by collaterals from persisting healthy axons or regenerating axons. We found innervation ratios near 1.00 in both the superficial pale portion, which exhibits type II predominance, and in the deep red portion of semitendinosus of pig which has extensive grouping of type I fibers. Therefore, the type grouping observed in pig skeletal muscle is not the result of multifiber innervation by subterminal axons, but, rather may be the manifestation of a unique motor unit topography.

In the work on nerve crush and reinnervation, hyperextension of the posterior limb and gross atrophy of the posterior limb muscles were both evident at 2 weeks following surgery. These clinical conditions were most severe at 2 to 3 months post nerve crush, but recovery was good by 5 to 6 months. The success of the nerve crush was also evident from other indications: (1) muscle contraction as elicited by stimulation of the nerve proximal to the site of crush was blocked whereas a contraction response was elicited by stimulation distal to the crush. (2) histological examination showed marked demyelination of the sciatic, tibial and peroneal nerves at 1 week post crush.

Histochemical study of muscle from pigs sacrificed over the time period of denervation and subsequent reinnervation resulted in the general conclusion that muscle fibers atrophied, became more homogeneous in properties, and then regained their distinguishing histochemical characteristics. One week after nerve crush, muscle fiber histochemistry was normal but by 10-11 weeks the differential staining of the NADH-TR and phosphorylase reactions was replaced by a non-specific staining. Differentiation was still obtained with the acid and alkaline ATPase reactions. Uniform atrophy of fibers within an entire fascicle, selective atrophy of fibers located peripherally in a fascicle and selective type II atrophy was observed in the semitendinosus, biceps femoris and gastrocmemius muscles. The broad spectrum staining was still present at 15 weeks but by 20 weeks differentiation of fiber types was again evident. A conspicuous grouping of fiber types, unlike that in normal pig muscle, was apparent. At 25, 27 and 31 weeks post nerve crush a more uniform size of fibers, conspicuous type groups and various degrees of fat infiltration were observed. There was no significant difference between reinnervated and control samples of superficial and deep semitendinosus for percentage of type I and type II fibers.

During the transformation from distinguishable fiber type to a homogeneous pattern and then back again to a differentiation of fiber types, the normal type grouping pattern of pig muscle was destroyed and replaced by a grouping pattern previously described to result from reinnervation in other mammalian muscle. The proportion of fiber types did not change even though the original spatial distribution was completely obliterated and even though the fibers lost their distinguishing characteristics during transformation.

In normal skeletal muscle, the FTIR approximates 1.00. In this study control muscle had a 1-7% incidence of branching of subterminal axons to innervate more than one muscle fiber and a 1-9% incidence of branching to form more than one end plate on a muscle fiber. In reinnervated muscle, both FTIR and ATIR were elevated, and significant-

ly ($P < .01$) greater in reinnervated compared to control muscle. FTIR ranged from 1.45 to 2.15 indicating extensive branching of the subterminal axons.

Overall, the results demonstrated that a crush of the upper sciatic and subsequent regrowth of the nerve caused a complete rearrangement of the original spatial distribution of fiber types in pig skeletal muscle. The results also show an association between collateral branching of axons and type grouping in reinnervated muscle. The percentages of fiber types was unchanged and we therefore concluded that regenerating axons were contained within their original endoneural tubes and that axons to both types of fibers possessed nearly equal potential for regeneration. The fact that muscle fibers dedifferentiated and then regained distinguishing histochemical properties after extensive collateral reinnervation strongly supports the concept and importance of a trophic influence. Our results also argue against the idea that certain stem lines of muscle fibers (i.e. type I and type II) attract preferentially a given type of axon. If this were the case then the normally occurring clusters of type I fibers should have been maintained. In the broad sense, our results add strong support to the idea that a neuron dictates the characteristics of a myofiber.

All indications point to a neuronal control of gene expression in individual fibers and therefore of muscle properties. The harnessing of such control for the benefit of animal production is a challenge for the future.

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BIOCHEMISTRY OF MUSCLE IN MALIGNANT HYPERTHERMIA

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Summary

1. Biopsies were taken from the Musculus longissimus of 5 Dutch Landrace pigs, designated to be susceptible and 5 littermate pigs designated to be non-susceptible to the Malignant Hyperthermia Syndrome with the halothane-test.
2. The activity of acetylcholine esterase was elevated in muscle homogenates from pigs susceptible to malignant hyperthermia. It was also found that the activity of this enzyme was higher in males compared to females.
3. The temperature coefficient (Q_{10}) of muscle fructose diphosphatse, if measured under in vivo conditions, is as high as 18. The extreme temperature dependence of this enzyme, that determines the rate of substrate cycling between fructose-6-phosphate and fructose-1,6-diphosphate in skeletal muscle, provides an explanation for the observed increase in substrate cycling in malignant hyperthermia. As the activity and allosterie properties of fructose diphosphatase are identical in muscle of susceptible and non-susceptible pigs, the increased sustrate cycling is the consequence of temperature elevation and not an inherited biochemical defect of malignant hyperthermia. As substrate cycling causes heat generation and ATP hydrolysis a vicious cycle may develop easily.
4. No differences could be detected between the contents of cyclic-AMP and cyclic-GMP in quick frozen muscle biopsies of susceptible and non-susceptible pigs. While the activity of low K_m phosphodiesterase was unchanged, a decreae in the basal activity of adenyl cyclase was observed in susceptible pigs.
5. The following working hypothesis is proposed for the initiation of malignant hyperthermia: susceptible pigs have an elevated level of cytosolic free Ca^{++}, probably due to increased turnover of acetylcholine and/or decreased cyclic C-AMP dependent Ca^{++} accumulation by intracellular membranes. Therefore only a slight elevation is needed, triggered e.g. by succinylcholine, stress or halothane, to bring the free Ca^{++} level above the threshold for muscle contraction; these contractions will be stronger and prolonged if compared to contractions in non-susceptible pigs.

Introduction

Malignant hyperthermia is an inheritable disease and expressed only when susceptible individuals are exposed to succinylcholine, potent inhalation anaesthetic, stress or a combination of these factors.
Despite numerous investigations the etiology of this syndrome is still obscure. It is probabaly triggered by an abnormally high level of Ca^{++} in the cytosol of skeletal muscle, which eventually may cause muscle contraction. It this situation remains a "vicious cycle" of hypermetabolism and elevation of temperature may develop.
The current study was designed to compare some biochemical parameters in muscle biopsies taken from reacting and non-reacting littermate pigs. Eventual differences found in the activity of enzymes e.g. acetylcholine esterase, fructose diphosphatase, phosphodiesterase and adenylcyclase or in the level of cyclic nucleotides, might possibly explain the accumulation of Ca^{++} in the cytosol and the generation of an excessive heat production in skeletal muscle of pigs susceptible to malignant hyperthermia.

Materials and methods

From three Dutch Landrace litters, tested at weaning for their susceptibility to halothane according to previously described methods (Eikelenboom and Minkema, 1974) an equal number of reacting and non-reacting pigs of the same sex were selected. When the animals weighed appr. 100 kg, 5 reactors and 5 non-reactors were anaesthetized through the intravenous administration of pentobarbitone sodium (Nembutal, Abbott Laboratories) until a moderate plane of surgical aneasthesia was obtained. A muscle biopsy of about 25 g. was removed from the longissimus muscle, using the surgical procedure described by Eikelenboom and Van den Bergh (1973), and immediately placed in isopentane cooled in liquid nitrogen. The sample was subsequently stored at - 90°C until analyses were performed.

Assay methods

Frozen muscle biopsies were used for the assay of choline esterase activities. 10 % homogenates were prepared by Polytron treatment in 0.25 M sucrose containing 10 mM tricine-KOH and 1 mM EDTA pH 7.4. A "soluble" and a "membrane" fraction were prepared by centrifuging the homogenate for 2 min. at 10.000 x g in the Eppendorf minilab centrifuge. Choline esterase activity was measured at 22°C with either 7 mM acetyl- or butyrylthiocholine in a medium containing 50 mM phosphate buffer

pH 7.7 and 4 mM $MgCl_2$. The thiocholine was reacted either continuously ("soluble" fraction) or discontinuously ("membrane" fraction) with DTNB and the reaction product was measured at 412 nm. A molar extinction coefficient of 13.600 at 412 nm was used for the anion produced.
Adenylcyclase was measured in homogenates prepared from freshly excised muscle as described by De Jonge (1975a). Phosphodiesterase was measured at 37°C in the same homogenates using 8 µM ^{3}H-cyclic-AMP. Fructose diphosphatase was measured in high speed supernatants of muscle homogenates using the standard incubation medium and various amounts of AMP as described by Van Tol (1975). Cyclic-GMP and cyclic-AMP were measured using binding proteins isolated from lobster tail muscle and bovine skeletal muscle respectively (for references see De Jonge, 1975b).
All values are given as mean ± SEM.

Results and Discussion

A. Acetylcholine esterase in skeletal muscle

Table 1 shows that membrane-bound acetylcholine esterase (AChE) activity is increased by 89 % in skeletal muscle of susceptible pigs, while the soluble activity is unchanged. As non-specific esterases may also contribute to the hydrolysis of acetylcholine in crude subcellular fractions we also measured the hydrolysis of butyrylcholine under the same conditions.
Table 2 shows that the latter activity, in contrast to AChE, resides mostly in the soluble fraction of skeletal muscle and is not changed significantly in susceptible pigs.
It may be concluded therefore that the membrane-bound activity of AChE is almost doubled in susceptible pigs. In addition it was found that the activity of AChE in muscle homogenates was higher in males compared to females (Table 3).
Incomplete relaxation of muscle after a standard dose of succinylcholine has been observed in patients subsequently developing the syndrome of malignant hyperthermia and an elevated AChE activity could contribute to this penomenon. If the high activity of AChE is and indication of increased turnover of acetylcholine, this could play a role in increasing the intra-cellular free Ca^{++} concentration, probably by increasing the permeability of the plasma membrane. A greater incidence of muscle pain an a more pronounced release of creatine phosphokinase after the administration of succinylcholine have been reported in female as compared to male subjects (Cooke et al., 1963; Perhoff et al., 1969). Although comparison between different species (man, dog and pig) is difficult, the observed relatively low AChE activity in female pigs, could play a role in muscle pain and damage induced by succinylcholine.

B. Skeletal muscle fructose diphosphatase

Hypermetabolism, as revealed by increased substrate cycling between fructose-6-phosphate and fructose-1,6-diphosphate in skeletal muscle, has been implicated in porcine malignant hyperthermia (Clark et al., 1973). As the maximal activity of phosphofructokinase is 10 times as high as that of fructose diphosphatase and because fructose diphosphatase will be partly inhibited by AMP already at concentrations less than 1 µM, it may be concluded that the activity of fructosediphosphatase will be rate-limiting for the substrate cycle under in vivo conditions. In previous studies, using purified fructose diphosphatase isolated from rabbit muscle, it was found that the inhibition by AMP decreases sharply with increasing temperature (Van Tol, 1975). This results in an apparent energy of activation of about 55 Kcal/mole and a temperature coefficient (Q_{10}) of 18, if the enzymatic activity is measured in the presence of Mg^{++} and small amounts of AMP, conditions likely to be present in vivo. It follows that a 3°C rise in temperature will give a 5-fold increase in the rate of ATP hydrolysis by the substrate cycle. As fructosediphosphatase isolated from skeletal muscle of man and pig are identical to the rabbit muscle enzyme (unpublished observation) the temperature effects as summarized above will be the same in man and pig.
Clark et al. measured increased substrate cycling in susceptible pigs, before as well as after induction of the hyperpyrexia by halothane. Susceptible pigs, however, already had elevated body temperature before the administration of halothane. If the 5-fold increase in cycling with every 3°C is taken into consideration, the increased substrate cycling in susceptible pigs can be explained by the higher body temperature. Table 4 shows that the maximal activity and the allosteric inhibition of fructosediphosphatase are unchanged in susceptible pigs. It is concluded that susceptible and non-susceptible pigs have muscle fructosediphosphatases with identical activities and allosterie properties. Once elevation of muscle temperature has occurred, the kinetics of this enzyme will induce ATP hydrolysis, hypermetabolism and more heat production by increased substrate cycling.

C. Metabolism of cyclic nucleotides in skeletal muscle

Table 5 shows that the levels of cyclic nucleotides measured in quick frozen muscle biopsies from susceptible and non-susceptible pigs are identical. As it is impossible to freeze-clamp pig skeletal muscle in situ, the muscle samples were excised under Nembutal anaesthesia and frozen as quickly as possible in isopentane cooled in liquid nitrogen. Still it will take about two seconds between excision and complete freezing. Therefore it is

not sure if the levels given in Table 5 are those existing in vivo.
We also measured the enzymatic activities of adenylcyclase and phosphodiesterase. The activity of the latter enzyme (low K_m from) was not changed in susceptible pigs as shown in Table 6. However, the basal activity of adenylcyclase (Table 7) was reduced significantly in susceptible pigs.
During the normal contraction-relaxation cycle in muscle, the free cytosolic Ca^{++} ions are removed by a cyclic AMP-dependent accumulation by the muscle membranes (Schwartz et al., 1976). The Ca^{++} accumulating properties of sarcoplasmic reticulum membranes are reported to be defect in patients with the trait for malignant hyperthermia (Kalow et al., 1970). This defect could also be detected in isolated sarcoplasmic reticulum from stress-susceptible pigs in the presence of halothane (Brucker et al., 1973). A decreased activity of adenylcyclase could result in a suboptimal level of cyclic AMP, thus impairing cyclic AMP dependent Ca^{++} accumulation by muscle membranes (sarcoplasmic reticulum and/or mitochondria).

D. Hypothesis

Susceptible pigs have an elevated level of cytosolic free Ca^{++} in muscle, probably due to increased turnover of acetylcholine and/or decreased cyclic-AMP dependent Ca^{++} accumulation by the sarcoplasmic reticulum and/or the mitochondria. Only a slight increase in the level of free Ca^{++} ions (by e.g. succinylcholine, halothane, stress of a combination of these factors) will surpass the threshold for muscle contraction. These contractions will be stronger and prolonged if compared to contractions in non-susceptible pigs. Contraction-induced elevation of muscle temperature will stimulate ATP hydrolysis by the substrate cycle between fructose-6-phosphate and fructose-1,6-diphosphate, and the vicious cycle of hypermetabolism, ATP hydrolysis and temperature elevation is born.

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Table 1. Acetylcholine esterase in pig skeletal muscle.

mU/gram ♀♀	Non-susceptible pigs	Susceptible pigs
"Soluble"	192 ± 8	211 ± 9[x]
"Membrane-bound"	120 ± 16	227 ± 10[xxx]

[x] statistically different from controls $p > 0.2$

[xxx] $p < 0.01$

Table 2. Butyrylcholine esterase in pig skeletal muscle.

mU/gram ♀♀	Non-susceptible pigs	Susceptible pigs
"Soluble"	86 ± 5	97 ± 7[x]
"Membrane-bound"	26 ± 7	47 ± 11[x]

[x] statistically different from controls $p > 0.1$

Table 3. Acetylcholine esterase in pig skeletal muscle.

mU/gram	Non-susceptible pigs	Susceptible pigs
Males	460 - 494	538 - 590
Females	312 ± 15[x]	438 ± 19[xx]

[x] statistically different from males $p<0.05$

[xx] $p<0.01$

Table 4. Fructose-1,6-diphosphatase in pig skeletal muscle.

	Non-susceptible pigs	Susceptible pigs
V_{max}(U/gram)	3.47 ± 0.10	4.00 ± 0.75
I_{50} AMP (μM)	2.4 ± 0.2	2.5 ± 0.1
Hill coefficient	2.5 ± 0.2	2.7 ± 0.1

Table 5. Cyclic nucleotides in pig skeletal muscle

pmoles/gram	Non-susceptible pigs	Susceptible pigs
cyclic-GMP	65 ± 3	68 ± 5
cyclic-AMP	492 ± 45	473 ± 30

Table 6. Phosphodiesterase in porcine skeletal muscle

mU/gram	Non-susceptible pigs	Susceptible pigs
With 8 μM cyclic-AMP	3.64 ± 0.21	3.81 ± 0.18
+10 mM theophilline	1.47 ± 0.10	1.64 ± 0.08

Table 7. Adenylcyclase in porcine skeletal muscle.

	Non-susceptible pigs	Susceptible pigs
Basal activity (mU/gram)	0.74 ± 0.06	0.58 ± 0.04[x]
Fluoride stimulation (%)	356 ± 11	425 ± 19[xx]

[x] statistically different from controls $p < 0.1$

[xx] $p < 0.05$

PRODUCTION OF LACTIC ACID IN DIFFERENT STRESS SITUATIONS IN PIGS [+]

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Summary

The flux of lactic acid through the extracellular compartment was studied in unanaesthetized pigs in different stress situations. The kinetic of lactate metabolism was investigated by continuous infusion of U-14C-L-lactate and periodically sampling of blood with implanted catheters. After adaption to experimental situations and persons the resting pigs (24 h after feeding) have a slow lactate metabolism similar to humans and dogs. In mild exertion (run of 3 km $\cdot h^{-1}$ for 20 min) the lactate turnover is significantly increased about 150% of resting value, in heavy exertion (run of 5 km$\cdot h^{-1}$ for 5 min) about 400% respectively. In a social stress situation the turnover of lactate is about 140% of the resting value, while an infusion of epinephrine (20$\mu g \cdot kg^{-1}$) caused a turnover increase to 300%. The highest lactate production (850%) is observed in pigs, which were immobilized by fixation with a nose loop.

In the best standardized exertion, in the steady state of run in a treadmill at 3 km$\cdot h^{-1}$, stress susceptible pigs show a significant higher lactate turnover as stress resistant pigs under the same conditions.

In both groups of pigs no feedback regulation of the plasma lactate level does exist, and the lactate level is highly correlated with the lactate turnover.

Introduction

Accumulation of lactic acid in extracellular fluid and muscle tissue is a typical finding in manifestation of stress in pigs (review: Marple & Cassens, 1973). Probably a lact-acidosis is directly responsible for cardiogenic shock in porcine stress syndrome (Muylle et al., 1968) and for changed meat quality. Therefore we have studied the kinetic of lactate and the regulation of lactate level in the extracellular fluid in different stress situations in pigs.

Material and Methods

15 stress resistant and 4 stress susceptible pigs (about 80kg b.weight) were selected from a landrace population by repeated Creatine-Kinase-Tests (Richter et al., 1973). In anaesthesia two polyvenylchlorid catheters were implanted into the atrium and the vena cava cranialis respectively for continuous infusion of radioactivity and for blood sampling. Electrodes for electrocardiography and a telemetric transmitter for registration of muscle pressure in the m.longissimus were also implanted (Maas, 1976). In the week following the operation the pigs were held alone and accustomed to experimental situations and persons. One week after the implantation procedure and 24 h after the last feeding (water ad lib.) the pigs were tested.

Blood samples were taken in 5 min intervals. A part was given into ice-cold perchloric acid immediately after sampling for estimation of concentrations of lactate, pyruvate and glucose and for detection of specific activity of U-14C-L-lactate (Jorfeldt, 1970) by enzymatic methods. The plasma concentrations of lactate, pyruvate and glucose were calculated by correction for packed cell volume. The turnover and the turnoverrate were calculated by a computer programme from the data of specific activity of lactate in blood and the constant of continuous infusion of labelled lactate (1,5$\mu Ci \cdot min^{-1}$).

Results

The number of pigs tested in different stress situations and the most interesting results are listed in the table 1.

Experiments with stress resistant pigs

- Rest: At rest the pigs were lying or standing in the treadmill-cage for one hour. The heart rate was 82 $\pm$ 9 $\cdot$ min^{-1} and the curve of muscle pressure showed only single peaks of muscle contractions.

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- Social stress: The customary pen of the experimental pig was divided diagonally with a fence and another unknown pig was introduced in the free part of the pen. Both pigs try to contact each other with the nose and run to and fro on the fence. The heart rate of the experimental pigs(N=7) increased significantly to 127±17·min^{-1} and the muscle pressure curve showed many peaks of activation similar to the activation pattern at run 3km·h^{-1}. The plasma levels of glucose,lactate and pyruvate were unchanged, but the packed cell volume was significantly increased to 0,37±0,03 l·l^{-1} as compared to resting values. At social stress of one pig the lactate turnover was found about 34µmol·min^{-1}·kg^{-1}.

- Run of 3 km·h^{-1} : After a resting period of one hour in the treadmill-cage the pigs had to walk steadily 1000 m in 20 min (3 km·h^{-1}). Only in the first 5 minutes of run a little increase of plasma lactate was observed. In the steady state from 5 to 20 min of walking the plasma lactate levels were of the same order as in rest, but the turnover and the turnoverrate of lactate were increased significantly to 150% of resting values(table 1). During walking heart rate was 185±33 ·min^{-1} and the muscle pressure curve showed continuously peaks of activation.

- Run of 5 km·h^{-1} : Immediately after walking 3 km·h^{-1} two pigs had to run 5 km·h^{-1} for 5 minutes. In this heavy work the plasma level of lactate increased significantly because the turnover was accelerated to 400% of resting value without a corresponding increase of turnoverrate. The heart rate (198 ±19 min^{-1}) was of the same order as at 3 km·h^{-1}.

- Immobilization : The forced immobilization of pigs by fixation with a nose loop immediately after a resting period was a heavy psychical and physical stress. All parameters of lactate metabolism were changed, but the turnoverrate was of the same order as in resting pigs (table 1). The heart rate was increased to 162±28 min^{-1} and the muscle pressure curve showed a pattern of tetanic activation followed by fatigue within 5 minutes.

- Infusion of Epinephrine : Intravenous infusion of 20 µg·kg^{-1} epinephrine in one minute produced changes of lactate metabolism during the next hour similar to heavy work, but the

Table 1 : Lactate metabolism in different stress situations

		stress resistant				stress susceptible
	rest	3 km·h^{-1}	5 km·h^{-1}	Immobilisation	Epinephrine	3 km·h^{-1}
Number of pigs(N)	15	5	2	8	6	4
Packed cell vol. l · l^{-1}	0,32±0,02	0,37±0,02 p<0,01[a]	0,39±0,01 + [b]	0,42±0,02 p<0,01[a]	0,38±0,02 p<0,01[a]	0,36±0,02 N.S. [c]
Plasma glucose mmol·l^{-1}	4,65±0,41	4,88±0,23 N.S.	4,83±0,15 N.S.	5,86±1,07 p<0,01	5,58±1,61 N.S.	4,73±0,48 N.S.
Plasma lactate mmol·l^{-1}	0,80±0,14	0,78±0,11 N.S.	2,73±1,17 +	11,53±2,10 p<0,01	5,73±0,92 p<0,01	1,10±0,05 N.S.
Lactate-pyruvate ratio	8,8±1,0	7,4±1,1 N.S.	21,7±9,3 +	36,4±6,7 p<0,01	18,5±2,7 p<0,01	10,3±1,7 p<0,05
Number of pigs(N)	7	5	2	2	2	4
Lactate Turnover µmol·min^{-1}·kg^{-1}	24,9± 6,9	37,1± 4,4 p<0,05	95,5±32,3 +	211,0±17,0 p<0,05	73,9±13,8 +	64,5±15,6 p<0,01
Lactate Turnoverrate min^{-1}	0,18±0,03	0,28±0,07 p<0,01	0,21±0,01 N.S.	0,13±0,02 N.S.	0,07±0,01 p<0,05	0,36±0,10 N.S.

a : Significance by analysis of variance between stress values and rest values of the same pigs
b:: Upper 95% intervall of rest value c: Significance by analysis of variance of stress values (3 km·h^{-1}) between stress resistent and stress susceptible pig groups.

turnoverrate was decreased significantly. The heart rate was highly accelerated in the first two minutes after infusion (234±4) and returned to the resting level during the following two minutes.

Experiments with stress susceptible pigs

A group of four stress susceptible pigs was tested at rest and by walking 3 $km \cdot h^{-1}$. Only in the steady state of walking these pigs showed a significant acceleration of lactate production (turnover) and consumption ability (turnoverrate) as compared to the stress resistant pigs in the same situation. No differences were detected between two stress susceptible and two stress resistant pigs tested at 5 $km \cdot h^{-1}$ and by immobilization.

Discussion

At rest the estimated blood parameters (PCV, plasma concentration of metabolites) and the turnover of lactate have low levels similar to humans and dogs (review: Lindena, 1975). Higher levels of plasma lactate in catheterized pigs at rest (Steinhardt et al., 1974; Kallweit et al., 1975) might be caused by feeding the pigs before or by stressing circumstances during experiments. In the resting state no differences of the investigated parameters are evident between stress resistant and stress susceptible pigs.

The most sensitive parameter in stress situations is the packed cell volume, which increases very quickly in all tested stress situations. The plasma level of lactic acid remains unchanged in mild psychical and physical stress situations (social stress or run of 3 $km \cdot h^{-1}$) because an increase of lactate production is accompanied by an increase of lactate metabolizing capacity (turnoverrate).

The relation between the lactate parameters is:

$$c = \frac{q}{k} \cdot V^{-1}$$

c = plasma concentration of lactate
q = turnover of lactate
k = turnoverrate of lactate
V = size of distribution compartment of lactate

In heavier work and stress situations (run of 5 $km \cdot h^{-1}$ or forced immobilization) the plasma lactate level is increased (table 1). Itis evident, that the energy consumption at heavy work is based on an increase of anaerobic glycolysis (lactate pyruvate ratio is also increased in plasma) and high production of lactic acid (turnover 400-800% of resting value), while the metabolizing capacity for lactate is not adequately adapted (turnover rate of the same order as at rest).

From the reaction of turnoverrate it is possible to conclude, that no feedback regulation of plasma level of lactate exists because the turnoverrate is neither correlated to plasma level ($r = -0,27$) nor to the turnover ($r = +0,17$) of lactate. But the turnoverrate is correlated significantly to the heart rate ($r = +0,62, p<0,001$). This may be explained by an increase of heart rate in the stress situation, which accelerates the cardiac output, the lactate oxidation in the heart muscle and possibly the lactate metabolizing mechanisms in liver and kidney (e.g. gluconeogenesis).

The lack of feedback regulation of lactate level in the extracellular fluid may be responsible for the high correlation between turnover and plasma level of lactate ($r = +0,92, p<0,001$) if all stress situations are considered except epinephrine infusion (see figure 1). The reaction of lactate metabolism after epinephrine infusion is different from all "physiological" stress reactions, because the turnoverrate is significantly suppressed (see table 1). This could result from vasoconstriction in different tissues and suppression of lactate metabolizing mechanisms.

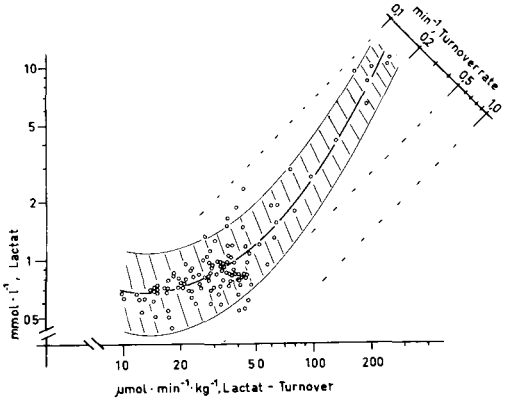

Figure 1: Relation between plasma level and turnover of lactic acid.
$r^2 = +0,85$
$\log y = 0,79(\log x)^2 - 1,81 \log x + 0,75$

The lactate metabolism in stress susceptible pigs is qualitatively the same, as in stress resistant pigs. But in the best standardized exertion, in the steady state of walking at 3 km $\cdot h^{-1}$, a significant higher lactate turnover (175%) and lactate pyruvate ratio is seen as compared to stress resistant pigs. It is evident, that stress susceptible pigs accelerate their anaerobic glycolysis more, than stress resistant pigs at the same exertion.

It is concluded from this study, that the high lactate production and following lactacidosis (correlation between plasma lactate level and pH of blood: $r = -0,87$, $p<0,001$) in pigs is caused rather by heavy exertion and tetanic muscle activation (run of 5 km$\cdot h^{-1}$, forced immobilization) than by voluntary mild motoric activity or psychical stress (run of 3km$\cdot h^{-1}$, social stress).

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A SEQUENCE OF PHYSIOLOGICAL CHANGES IN AN EXPERIMENTALLY ATTENUATED FORM OF THE MALIGNANT HYPERTHERMIA SYNDROME

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Summary

Halothane-susceptible pigs which were anaesthetised with pentobarbitone and then subjected to halothane slowly developed rigidity of the skeletal muscles after 1 h. During the interval between the administration of halothane and the development of rigidity there was a steady loss of adenosine triphosphate and creatine phosphate from m. semitendinosus which was accompanied by formation of lactate. The results suggest that loss of ATP may be an early intracellular event in the adverse response to halothane and that it may occur primarily in red rather than white myofibres.

Introduction

The malignant hyperthermic syndrome (MHS) develops in susceptible pigs following the inhalation of the anaesthetic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane). The syndrome often develops rapidly and consists of a complex of symptoms - extreme rigidity of the skeletal muscles, hyperthermia, a rise in oxygen consumption by the tissues, elevation of the levels of K^+, Ca^{2+}, Mg^{2+}, lactate (LA) and catecholamines in blood, hypercarbia, tachycardia and cardiac arrythmia. The mortality rate is high and death follows from cardiovascular failure. It is difficult to observe a sequence of physiological changes in acute MHS but recent studies by Gronert and Theye (1976) and Lucke et al. (1976) indicate that one of the earliest indications of the development of MHS is a rise in blood lactate. Such an increase in anaerobic glycolysis, at a time when the oxygen consumption of muscle is high, suggests that an increased hydrolysis of adenosine triphosphate (ATP) may be a primary intracellular event which stimulates both anaerobic glycolysis and oxidative metabolism. In the work reported in this paper, an attempt was made to study the changes in the energy phosphate ($\sim$P) and LA content of muscle during an attenuated or slowly-developing form of MHS before the onset of rigidity or hyperthermia.

Results and discussion

Pietrain pigs which had developed rigidity of the hind limbs and a rise in body temperature in less than 5 min following the commencement of halothane anaesthesia (5% halothane in O_2) were classed as MHS-susceptible and were used as experimental animals. Preliminary observations showed that when pigs were subjected to full surgical anaesthesia with pentobarbitone they did not develop acute MHS when halothane was administered to continue anaesthesia. Indeed, these animals did not react up to 30 min. on halothane. Four MHS-susceptible pigs were then anaesthetised with pentobarbitone, intubated and maintained on halothane for longer periods. The E.C.G. and blood pressure (via a cannula in the carotid artery) were recorded. Biopsy specimens of the predominantly red and white fibre areas of m. semitendinosus were taken under pentobarbitone anaesthesia and at intervals following the administration of halothane until a rigid extension of the hind limbs occurred.

The development of muscle rigidity was delayed for about 1 h following pentobarbitone anaesthesia (the actual times were 58, 70, 60 and 65 min). During this interval there was a fairly steady fall in arterial blood pressure from about 125 initially to 75 mm Hg around the time when rigidity developed. The heart rate varied between the four pigs but in general tachycardia did not develop until rigidity was evident. It is possible that cardio-acceleration initially was a reflex response to the fall in blood pressure. Body temperature rose in one animal only up to the development of rigidity (Table 1).

Table 1. Temperature change in attenuated MHS.

	Temperature (°C)			
Animal No.	1	2	3	4
Before halothane	-	38.2	37.2	39.1
At rigidity (60 min)	36.6	38.8	37.0	39.1

An examination of the CP and lactate content of biopsy specimens showed that changes had occurred in these parameters in skelebal muscle shortly after the administration of halothane and before the development of rigidity, tachycardia or hyperthermia (Table 2).

Table 2. Changes in CP, ATP and LA during attenuated MHS.

Time on halothane (min)	CP (μmol/g)		ATP (μmol/g)		LA (μmol/g)	
	R	W	R	W	R	W
0	15.6	20.0	6.8	7.4	9.1	11.1
15	8.5	18.8	5.3	7.0	9.5	10.1
30	9.1	15.4	6.1	6.6	15.6	15.4
40	6.6	14.6	6.4	6.9	12.2	9.0
60	3.5	11.3	5.2	6.6	8.9	11.9
75	-	-	4.5	6.2	19.9	20.6

R = red, W = white myofibres

A sharp fall (46%) occurred in the CP content of the red myofibres after 15 min on halothane; the fall in white myofibre CP was much less (6%). Over the period of 60 min, CP appeared to disappear more rapidly from red than from white myofibres and at rigidity the latter still retained somewhat more than 50% of the initial concentration of this energy phosphate. Since CP is the immediate source of P for the resynthesis of ATP in muscle, the fall in CP following the administration of halothane presumably reflects an increased rate of ATP utilisation. The increased use of ATP also stimulated anaerobic glycolysis and production of LA. After an initial fall, the ATP levels rose in both myofibre types after about 30 - 40 min. This rise suggested that resynthetic mechanisms were now just outbalancing hydrolytic at least temporarily. At this time also there was a fall in the LA content of the muscle. The transient rise in ATP and fall in LA are consistent with the observation by Gronert and Theye (1976) that an increase in O_2 consumption by the tissues occurred about 30 min after the administration of halothane to MHS-susceptible pigs under thiopentone anaesthesia but declined again terminally. Lucke et al. (1976) also reported that a steep increase in O_2 consumption occurred in pigs exposed to both halothane and suxamethonium.

The results reported in this paper suggest that a loss of energy phosphate, which stimulates anaerobic glycolysis in spite (presumably) of a high consumption of O_2 by the tissues, is an early intracellular event which precedes the development of clinical symptoms of MHS such as rigidity of the skeletal muscles, hyperthermia and tachycardia. In the presence of a high consumption of oxygen by the tissue the loss of ATP would appear to be due to an increased rate of utilisation rather than a reduced rate of resynthesis. Elevated levels of myoplasmic Ca^{2+} in MHS might be an event secondary to loss of ATP since this energy phosphate is required to fuel the Ca^{2+} pump of the sarcoplasmic reticulum. The loss of ATP might also result from changes in membrane permeability that cause alterations in the ionic strength of the intracellular fluid. Mothersill & McLoughlin (1975) reported that the acto-myosin ATPase of red myofibres was more sensitive to fluctuations in ionic strength than was that of white. The observations reported in this paper indicate that the loss of energy phosphate which follows the administration of halothane is more marked in red than in white myofibres and suggest that halothane-induced rigidity may result from a defect primarily in red rather than in white myofibres.

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THE PREVENTION OF ACUTE MALIGNANT HYPERTHERMIA IN HALOTHANE-SENSITIVE PIETRAIN PIGS BY LOW DOSES OF NEUROLEPTIC DRUGS

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Summary

Intramuscular injection of low doses of the neuroleptic drugs, azaperone, spiperone and haloperidol, prevented the acute development of the malignant hyperthermic syndrome (MHS) in halothane-sensitive Pietrain pigs. The effective dose of spiperone was one twentieth that of the other two drugs. Following premedication with azaperone, susceptible pigs under full surgical anaesthesia with halothane slowly developed a muscular rigidity which was not accompanied invariably by hyperthermia. Control animals which were not treated with neuroleptics showed a positive reaction within 2 to 3 min following inhalation of the anaesthetic.

Introduction

Azaperone (4-fluoro-4-[4-(2-pyridyl)-piperazinyl] butyrophenone) has been used in the pig to reduce the incidence of aggressive behaviour and to protect against adverse reactions to physiological stress (Symoens, 1970). Administered ante-mortem, the drug lowers the temperature of the carcase and delays the onset of rigor mortis (Devloo et al., 1971; Oldigs & Unshelm, 1971). It also raises significantly the high energy phosphate (νP) content of skeletal muscle in both the rabbit and pig (McLoughlin & Heffron, 1975). Recently, Somers et al. (1976) found that skeletal muscle from halothane-sensitive Pietrain pigs which had been premedicated with azaperone and anaesthetised with pentobarbitone exhibited the slow rates of ν P splitting and anaerobic glycolysis in vitro that are characteristic of resting muscle from stress-resistant breeds of pig and species such as the rabbit and dog. As a result of the latter observation, the effect of azaperone on the onset of the acute hyperthermic syndrome (MHS) was studied. The investigation also included two other neuroleptic drugs, haloperidol (4-[(p-chlorophenyl)-4-hydroxypiperidino]-4-fluorobutyrophenone) which is widely used in the therapy of behavioural disorders in man and spiperone (8-[3-(p-fluorobenzoyl)propyl]-1-phenyl-1, 3,8-triazospiro-[4,5]-decan-4-one).

Results and discussion

Sensitivity to the anaesthetic halothane (2-bromo-2-chloro-1,1,1,-trifluoroethane) was detected by permitting Pietrain pigs to breathe the anaesthetic at a concentration of 5% in oxygen delivered at a rate of 2 l/min. The concentration of the anaesthetic was adjusted if and when the rate and depth of respiration required. The rectal temperature was recorded throughout the procedure. A rigid extension of the hind limbs and a rise in body temperature were taken to indicate susceptibility to halothane. When the symptoms of MHS appeared, halothane was immediately discontinued although in spite of this precaution several animals died during testing. An animal was considered to have reacted positively, i.e. was susceptible to MHS, when symptoms developed within 5 min following the commencement of anaesthesia. This type of test clearly has limitations but it was useful in screening drugs to see whether or not they influenced the onset of the acute syndrome. Furthermore, all previous Pietrain pigs belonging to the herd used in these experiments and which had been subjected to halothane anaesthesia had shown an explosive development of MHS within 2 to 3 min (McLoughlin & Mothersill, 1976). The action of the neuroleptic drugs was studied by injecting the drug intramuscularly and subjecting the animal to halothane 30 min later. Control pigs were included in each experiment. The drugs azaperone, spiperone and haloperidol were obtained from Jannssen Pharmaceutica, Belgium.

Azaperone

The effect of the concentration of azaperone on the appearance (+) or non-appearance (-) of symptoms of MHS are given in Table 1. A high concentration of the drug (8mg/kg) prevented the onset of MHS in all animals tested. The lower dose limit which effectively prevented MHS was in the region of 0.15 to 0.2 mg azaperone/kg; lower concentrations were ineffective.

Table 1. Effect of azaperone on the acute development of MHS.

Dose	No.	Reaction	
		-	+
8.0 mg/kg	12	12	0
0.5 mg/kg	6	6	0
0.2 mg/kg	8	7	1
0.15 mg/kg	8	6	2
70 µg/kg	3	0	3
15 µg/kg	2	0	2
Controls	15	0	15

- = no reaction; + = symptoms of MHS

A group of animals which had been premedicated with azaperone (8 mg/kg) were exposed to halothane at intervals of 2, 3, 6, 19 and 25 h after the treatment. No reaction occurred up to and at 19 h. A positive reaction was obtained at 25 h.

Spiperone

The neuroleptic spiperone was strikingly effective in preventing the onset of acute MHS at low dose levels (Table 2). A dose of approximately 10 µg/kg prevented the development of symptoms in 5 of 6 pigs. Three pigs which were given a dose of 0.5 mg/kg did not give a positive response to halothane 20 h later.

Table 2. Effect of spiperone on the acute development of MHS

Dose	No.	Reaction	
		-	+
300 µg/kg	1	1	0
75 µg/kg	1	1	0
15 µg/kg	1	1	0
10 µg/kg	6	5	1
8 µg/kg	1	0	1
5 µg/kg	3	0	3
Controls	3	0	3

Haloperidol

This neuroleptic also prevented the development of the symptoms of MHS under the testing conditions employed (Table 3). The effective doses were similar to those for azaperone.

Table 3. Effect of haloperidol on the acute development of MHS.

Dose	No.	Reaction	
		-	+
0.5 mg/kg	5	5	0
0.2 mg/kg	2	2	0
0.15 mg/kg	1	1	0
0.1 mg/kg	2	0	2
Controls	2	0	2

Three MHS-susceptible pigs were premedicated with azaperone (8 mg/kg), anaesthetised with halothane, intubated and maintained under full surgical anaesthesia with halothane. Pig 1 developed muscular rigidity after 55 min. Body temperature was 38.8°C at rigidity and did not rise above this level during a further 30 min inhalation of halothane. In contrast to this, the onset of rigidity in pig 2 (45 min) and pig 3 (55 min) was accompanied by a rise in temperature (pig 1, 38.8 to 40.9°C; pig 2, 37.9 to 39.4°C) during the period of anaesthesia. In each pig cardioacceleration was associated with the development of muscular rigidity.

A further 3 pigs which previously had developed acute MHS following the inhalation of halothane were premedicated with spiperone (0.4 mg/kg) and subjected to full surgical anaesthesia. Pig 1 did not develop muscular rigidity nor hyperthermia during a 95 min period under anaesthesia. At this time, the rectal temperature was still 37°C and the muscles appeared to be fully relaxed. While pig 2 did not develop muscle rigidity during 85 min under halothane, the body temperature, which had been 38°C at 65 min, rose to 40°C by 85 min. A marked rigidity of the hind limbs developed in pig 3 after 60 min anaesthesia. The body temperature was 37°C at this time but rose by 1°C during the next 20 min. The inhibitory action of

spiperone was greater than that of azaperone since it was used at 1/20 the dose of the latter drug. The effectiveness of the drugs may be related to their neuroleptic potency. Spiperone is a derivative of the 4-anilinopiperidines which are the most potent neuroleptics.

The results indicate that azaperone, spiperone and haloperidol, given intramuscularly and in small quantities, markedly delay or attenuate the response of susceptible animals to halothane. It is highly unlikely that the drugs have a direct influence on skeletal muscle and it is this tissue which is considered to be the primary site of halothane action in susceptible individuals. The findings suggest that central neural mechanisms which normally control stress-induced physiological responses may be involved in the development of the complex of symptoms which characterise MHS. The biogenic amines dopamine, noradrenaline and serotonin appear to play an inhibitory role in the regulation of the hypothalamo-hypophyseal-adrenocortical system. It is possible that neuroleptic drugs antagonistic to the action of the monoamines might facilitate the activation of this neuroendocrinological axis and thus permit the adequate response to stress which occurs in normal animals but not in the Pietrain pig.

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DISTRIBUTION AND VARIATION OF CREATINE KINASE AND LACTATE DEHYDROGENASE IN DIFFERENT GROUPS OF BELGIAN PIGS

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Summary

Blood samples of more than 600 pigs were analysed on their content of CK, LDH and LDH-isoenzymes. These pigs were purebred Belgian Landrace, Piétrain and Large White, crossbreds between these breeds and Belgian Landrace which suffered from "back muscle necrosis". The amount of CK, LDH total and the LDH5-isoenzyme show significant skewness and kurtosis. For these values, a log. transformation yields good results in order to obtain a statistical normal distribution. An arcsinus transformation had to be used for the LDH5-isoenzyme percentage. Large White is less stress susceptible than Belgian Landrace and also reduces stress susceptibility in crossbreds. For the"back muscle necrosis" groups, some values were quite different from the normal BL. Significant correlations are found between the different parameters studied.

Using the hypothesis of single inheritance and recessivity of stress susceptibility, a gene frequence for stress susceptibility of .6 in BL and of .4 in LW was calculated.

1. Introduction

Stress susceptibility has become a major problem in pig rearing. The 2 main reasons for economic losses are : death animals on the farm and during transport and losses due to P.S.E. in the slaughter house.

Because a high frequency of stress susceptibility appears to exist among Belgian swine, studies on this subject were planned. The first object of the research program was to carry out a pilot study. As parameters for stress susceptibility, the enzymes creatine kinase and lactate dehydrogenase were choosen according to Hessel de Heer (1968), Bickhardt (1969,1970), Allen and Patterson (1971) and several others.

This paper deals with the study of the statistical distribution of the parameters and the correlation that may exist between them. A first approach on the genetics of stress susceptibility based on these results was performed.

2. Literature

Concerning the problem of the statistical distribution, we can mention the work of Richter et al. (1973) in which especially the CK-content was studied.

With regard to the genetics of stress susceptibility, research work was done by Christian (1972), Richter et al. (1973), Flock et al. (1974), Ollivier et al. (1975) and Eikelenboom et al. (1976).

3. Material and methods

3.1. Animals

The blood samples were collected from pigs which were used in a crossbreeding experiment and from pigs whose parents are from the BL, but suffering from "back muscle necrosis". The different groups are given in table 1.

Table 1. Number of animals in the different groups.

Belgian Landrace (BL)	189
Piétrain (P)	16
Large White (LW)	105
BL x LW	35
BL x LW x P	40
BL x LW x BL	92
BL x Back Muscle Disease (BMD)	84
BMD x BMD	88

3.2. Sampling

Blood samples were taken directly from the v.jugularis when the pigs weighted 25 kg. A first sample was collected early in the morning,a second

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one directly after a walk of 5 minutes at 6 Km/hour and a third one 4 hours after the walk. In the serum of the first sample the content of CK, LDH, LDH-isoenzymes and lactate was measured. In the second sample only the lactate content and in the third one the CK, LDH and the LDH-isoenzymes were determined.

3.3. Analytical methods

The CK, LDH and the lactate contents were determined with the spectophotometer according the methodes indicated by Boehringer, Mannheim. A filter of Hg 366 nm was used. For the LDH-isoenzymes, electroforesis on cellulose acetate (Gelman) was performed. The quantitation of the isoenzymes was done colorimetrically with a filter Hg 578 nm.

3.4. Statistical analysis

The generally known formulas are used for the calculation of the mean, the standard deviation, the skewness, the kurtosis and the correlation coefficients. The differences between the various groups are studied with an analysis of variance, followed by a Duncan-Kramer-Test.

4. Results and discussion

4.1. The statistical parameters

The mean ($\bar{x}$), standard deviation (s), skewness (g1) and kurtosis (g2) of the pooled real and transformed results are given in table 2.

From table 2 it is clear that the results of CK are not distributed normally but that a logaritmic transformation presents a good alternative. The same is true, although to a somewhat smaller extent, for the LDH-total and for the LDH5 quantity. The lactic acid quantity does not need transformation. Finally the LDH-% can be transformed with good results by the arc. sin. $\sqrt{\text{perc.}}$ transformation.

Generally spoken the transformation yields a better result for the parameters prior to stress than after stress. For some of these deviations an explanation is proposed.

4.2. Comparison of the different groups

Since it is impossible to give all the results in detail in the present report, only log CK and transformed LDH5-% after stress are printed out in table 3a and 3b. The figures mentioned after the results indicate the statistical different groups.

Table 2. Mean ($\bar{x}$), standard deviation (s), skewness (g1) and kurtosis (g2) of the different parameters before and after transformation.

Character	$\bar{x}$	s	g1	g2
1*	352,8	328,5	2,76	11,55
2	2,403	0,359	-0,098	0,118
3	1710	1290	1,329	1,735
4	3,098	0,376	-0,724	0,803
5	610,0	225,9	0,928	3,297
6	2,753	0,175	-0,854	2,028
7	1306	1130	3,790	20,89
8	3,020	0,270	0,925	0,942
9	79,1	30,9	0,530	0,647
10	1,859	0,207	-2,173	13,66
11	115,8	41,5	0,316	-0,388
12	2,033	0,169	-0,529	0,034
13	7,76	6,97	1,358	2,603
14	14,08	8,37	-0,017	-0,277
15	23,03	12,31	0,412	0,853
16	27,55	9,55	-0,464	0,833
17	61,30	60,43	2,718	12,71
18	1,602	0,428	-0,391	-0,057
19	377,0	524,5	4,991	36,80
20	2,310	0,526	-0,594	0,678

* : 1: CK before stress; 2: log CK bef 3: CK after stress; 4: log CK aft; 5: LDH bef; 6: log LDH bef; 7: LDH aft 8: log LDH aft; 9: lactate bef; 10:log lactate bef; 11: lact aft; 12: log lact aft; 13: LDH5 % bef; 14: arc sin $\sqrt{\%}$ LDH5 bef; 15: LDH5 % aft; 16: arc sin $\sqrt{\%}$ LDH5 aft; 17: LDH5 quant bef; 18: log LDH5 quant bef; 19: LDH5 quant aft; 20: log LDH5 quant aft.

Table 3a. Mean and standard deviation for log CK after.

Group	$\bar{x}$	s	
1. P x P	3,261	0,2981	(8,7,6)
2. BMD x BMD	3,258	0,2565	(8,7,6)
3. BL x BL	3,192	0,3165	(8,7,6)
4. BL x BMD	3,140	0,2916	(8,7,6)
5. BL x LW x P	3,129	0,4077	(8)
6. BL x LW x BL	2,997	0,4304	(8)
7. BL x LW	2,987	0,3329	
8. LW x LW	2,863	0,4278	

The other results have shown that the LDH total and the LDH5 quantity, nearly give the same sequence as the LDH5 %. From these results and from the data presented in tables 3a and 3b, it can be concluded that the LW has a

Table 3b. Mean and standard deviation for arc sin $\sqrt{LDH5\ \%}$ after.

Group	$\bar{x}$	s	
1. P x P	32,01	5,57	(8—— 4)
2. BMD x BMD	31,70	7,67	(8—— 3)
3. BL x LW x P	30,38	11,17	(8—— 5)
4. BL x BL	30,05	7,82	(8—— 5)
5. BL x LW x BL	26,01	9,47	(8—— 6)
6. LW x LW	24,98	9,54	(8,7)
7. BL x BMD	22,72	9,48	
8. BL x LW	22,03	11,81	

good influence. The CK and the LDH-values increase when more BL-blood comes in a combination. The Piétrain still gives higher enzyme levels. The back muscle groups give somewhat strange results : the BMD x BMD group is very similar to the BL group, but the BL x BMD group nearly equals the BL group for the CK, but not for the LDH. The BMD x BL group has indeed one of the lowest LDH levels. An explanation for this very unexpected phenomenon cannot be given.

4.3. The correlation between the studied characters.

The correlation coefficients for the pooled groups are given in table 4.

Table 4. Correlation coefficients between the studied characters (after stress).

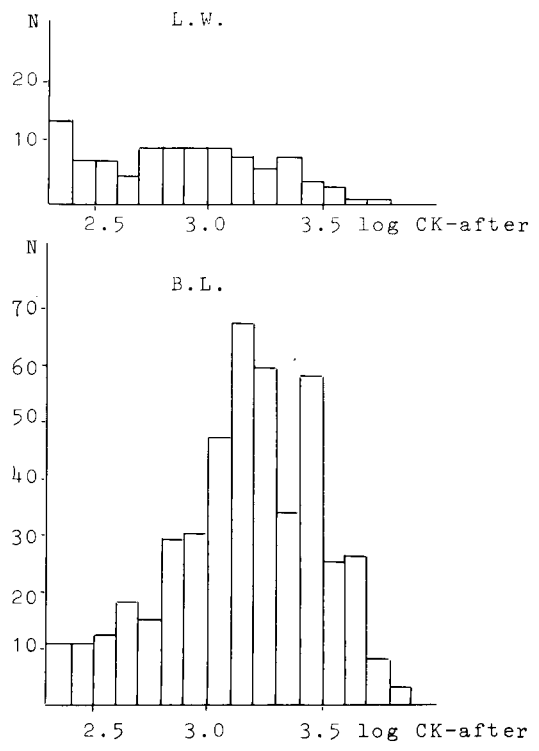
Characters	r	s_r
Log CK aft.		
Log LDH aft.	0,65	0,03
Log CK aft.		
Lact. Acid aft.	0,26	0,04
Log CK aft.		
Arc.sin. $\sqrt{LDH5}$ % aft.	0,61	0,03
Log LDH aft.		
Lact. Acid aft.	0,13	0,04
Log LDH aft.		
Arc. sin. $\sqrt{\%\ LDH5}$ aft	0,59	0,03
Lact. Acid.		
Arc. sin. $\sqrt{LDH5\ \%}$ aft.	0,14	0,04
Log CK aft.		
Log LDH5 quant aft.	0,68	0,03
Log LDH aft.		
Log LDH5 quant aft.	0,85	0,02
Log LDH5 quant aft.		
Arc. sin. $\sqrt{LDH5\ \%}$ aft.	0,90	0,02
Log LDH5 quant aft.		
Lact. acid. aft.	0,12	0,04

From table 4 can be concluded that there is a very good correlation between the LDH5 quantity and the LDH5 %. With the LDH total, the correlation of the LDH5 quantity is higher than that of the LDH5 %. The correlation between CK and LDH reaches the .6 value. We find that this is not high enough to drop some of these parameters. The definite choice between the LDH5 quantity and the LDH5 percentage has to be postponed because we do not have results between these parameters and the degree of PSE. Finally, there is a low but significant correlation between the CK, LDH total and LDH5 isoenzyme on the one side and the lactic acid content of the serum on the other side. This means that the absolute value of the enzymes has to be seen in relation to the "stress dose".

4.4. Variation and heritability

We have seen earlier that the statistical transformation of the CK and the LDH5 give not as good results for the after stress values than for the before stress values. In fig. 1 the histogram of the CK values after stress is reproduced for all the BL groups and for the LW group.

Fig. 1. Frequency distribution of the CK-after stress.



From fig. 1 it appears that there is not a normal but rather a three-topped distribution for this parameter. That type of distribution can be obtained with characters which follow a monofactorial heredity. Making groups as indicated in table 5 and using the Hardy-Weinberg formula, a scattering of the gene frequency of stress susceptibility was performed. The results are given in table 5.

Table 5. Obtained and calculated gene frequency distribution on the hypothesis of monofactorial heredity.

			p^2	2 pq	q^2
BL	Log CK aft.	obt.	67	232	154
		calc.	79	220	154
	$q = 0{,}583$; χ^2 = N.S.				
	Arc.sin. LDH5 $\sqrt{\%}$ aft.	obt.	55	209	189
		calc.	57	207	189
	$q = 0{,}646$; χ^2 = N.S.				
	Log LDH5 aft.	obt.	72	207	170
		calc.	67	212	170
	$q = 0{,}615$; χ^2 = N.S.				
LW	Log CK aft.	obt.	43	44	17
		calc.	37	50	17
	$q = 0{,}404$; χ^2 = N.S.				
	Arc.sin.LDH5 $\sqrt{\%}$ aft.	obt.	58	29	17
		calc.	37	50	17
	$q = 0{,}404$; χ^2 = $p < 0{,}01$				
	Log LDH5 aft.	obt.	23	64	16
		calc.	38	49	16
	$q = 0{,}396$; χ^2 = $p < 0{,}01$				

Limits of the groups : log CK aft. a) $< 2{,}8$ b) 2,8-3,3 c) $> 3{,}3$; log LDH5 quant. a) $< 1{,}9$ b) 1,9-2,5 c) $> 2{,}5$; arc.sin. LDH5 $\sqrt{\%}$ a) < 18 b) 18-30 c) > 30.

Gene frequencies for both the BL and the LW group agree very well even though some significant deviations are found, possibly due to the small number of LW-pigs or to the somewhat empirical limitation of the groups. It is clear that the experiments have to be continued in order to verify these results.

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SCREENING TESTS FOR IN VIVO DETECTION OF STRESS-SUSCEPTIBILITY OF SWINE UNDER FIELD CONDITIONS

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Summary

In a running programme in a SPF-breeding farm 27 pigs have been tested at about 30, 50 and 90 kg live weight for stress-susceptibility by the determinations of creatine-kinase (CK) and other parameters in two to three successive blood samples. The stress consists of catching the animals, fixation and blood collecting. The second and third samples have been taken about one hour and four hours, respectively, after the first ones. There were significant increases of log CK from the first to the second and from the second to the third samples ($P < 0.001$). In addition to the in vivo CK-tests meat quality characteristics have been determined and correlations calculated.

Introduction

In the last years growth rate and meatiness of fattening pigs were steadily increasing. At the same time also the percentage of stress-susceptible pigs that die under the influence of excitement or that exhibit pale, soft and exudative (PSE) meat increased. The aim of research is to find suitable parameters for detecting highly stress-susceptible pigs already in early life.

We decided to start with a rather large scale of blood analyses. Relying upon experiments done elsewhere (Bickhardt, 1971; Kraft, 1975; Schmidt et al., 1970), the following determinations have been chosen with the methods used in parentheses: haematocrit and haemoglobin (cyanmethaemoglobin) in blood, creatine-kinase (colorimetric Sigma test), urea-N (urease), glucose (GOD), inorganic phosphorus (molybdeniumblue), calcium (atomic absorption spectrometry), protein (biuret) and alkaline phosphatase (nitrophenol) in serum.

We are testing whether the collecting of blood by itself without specially exercising the animals is a sufficient stressor to detect increased stress-susceptibility. Three collections are carried out at a live weight of about 30 (period A), 50 (period B) and 90 kg (period C). The sampling of the blood from an ear vein or V. cephalica starts at about 9.30 a.m. (sample 1). A second and in some cases a third sample are taken about one hour (sample 2) and four hours (sample 3), respectively, after the first one in order to compare possible short and long time effects after the first stress.

All pigs examined are reared under similar conditions. The pigs are slaughtered at about 100 kg live weight, whereby an additional blood sample is taken during exsanguination. As stress-susceptible pigs show an increased incidence of PSE-meat, the meat quality is assessed by measuring pH 45 min p.m. of M. **adductor** (pH-meter Polymetron) and reflectance of M. longissimus dorsi 24 hours p.m. (Unigalvo, Evans Electroselenium Ltd.). High reflectance values indicate a lighter color.

The left side of each carcass is divided into wholesale cuts. The lean meat parts of M. longissimus, ham and shoulder are expressed in percentage of the carcass weight.

Results and discussion

27 pigs have hitherto been tested: 12 pigs from three Landrace litters in fall 1975 and 15 pigs from one Landrace and one Landrace x Hampshire litter in spring 1976.

From all the parameters tested the CK values gave the most consistent results with regard to the different samples in the three weight periods and in comparison with meat quality characteristics (tables 1 and 2).

Table 1. Log CK of the different weight periods and blood samples.

period/ sample	n	mean	SD
A/1	27	1.584	0.229
/2	27	1.911	0.335
/3	15	2.088	0.403
B/1	15	1.538	0.153
/2	15	1.653	0.179
/3	15	1.803	0.270
C/1	27	1.612	0.235
/2	27	1.701	0.271
/3	27	1.903	0.400
slaughter	27	1.937	0.210

The values from the first samples did not differ significantly between the periods ($P > 0.05$). In all periods the second and third samples revealed a significant increase of log CK ($P < 0.001$). This increase was significantly higher ($P < 0.01$) for period A as compared to periods B and C. Most of the pigs having the highest CK values in period A showed also the highest CK response in the later periods and at slaughter. It seems that succeeding blood sampling provoked a more pronounced response in young than in older pigs.

Table 2. Correlation coefficients of log CK to meat quality characteristics.

period/ sample	n	pH 45 min	reflectance %	lean meat %
A/1	27	-0.44*	0.59**	0.20
/2	27	-0.38*	0.38*	0.41*
/3	15	-0.18	0.33	0.53*
B/1	15	-0.35	0.41	0.28
/2	15	-0.58*	0.66**	0.48
/3	15	-0.58*	0.75**	0.54*
C/1	27	-0.44*	0.42*	0.36
/2	27	-0.46*	0.44*	0.39*
/3	27	-0.46*	0.41*	0.41*
slaughter	27	-0.48*	0.57**	0.30

* $P < 0.05$, ** $P < 0.01$

In view of the fact that stress-susceptible pigs may develop either PSE, normal or dark, firm and dry meat depending on strength and duration of stress (Topel et al., 1973), the pH 45 min and reflectance were fairly good correlated with log CK of nearly all samples. From the 27 pigs 10 had a pH 45 min between 6.9 and 6.6, 12 between 6.5 and 6.2 and 5 below 6.2. The correlation coefficient of pH 45 min to reflectance was -0.66.

It is of special interest that the correlation coefficients of log CK to pH 45 min and reflectance calculated for the first samples were as high in period A as in the following periods. Further it should be emphasized that with the exception of period B, comprising 15 animals only, the second and third samples did not reveal higher correlation coefficients as compared to the first samples.

Lean meat percentage was only significantly correlated with the second and third samples' log CK. The correlation coefficients of lean meat to pH 45 min and reflectance were -0.34 and 0.26, respectively.

Our preliminary results indicate that by determination of CK in two successive blood samples, or perhaps in one sample only, it may be possible to detect highly stress-susceptible pigs already at the beginning of the fattening period. For a definite decision further investigations are needed.

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FACTORS WHICH MAY AFFECT CK ESTIMATIONS IN THE PIG

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Summary

Various factors influence the correlation between CK activity in plasma and both stress susceptibility (SS) and inferior meat quality (pale, soft, exudative muscle or PSE). In all pigs it has been demonstrated that CK activity is affected by the methods of sample collection and handling of animals, by the use of various drugs including antibiotics, anaesthetics and tranquilisers, by the relative tissue concentrations of the enzyme and by its rate of release from tissue and its persistence in plasma

Behavioural factors may also influence the occurrence of SS and PSE. Because of the large number of variables, any single test for SS or PSE will be relatively inefficient for prediction and probably incapable of significant improvement; in order to evaluate any predictive test all the above relevant factors must be considered.

Introduction

The pig industry selects stock with improved food conversion efficiency and an increased carcase lean meat content. However these desirable characteristics may be associated with susceptibility to the acute stress syndrome (SS), acute back muscle necrosis and production of inferior quality meat (PSE) which in certain breeds or strains of pigs reduce their commercial profitability. Methods of predicting this susceptibility on farms would therefore be useful for breeding and selection programmes, and ideally should accurately predict susceptibility early in life in order to avoid the cost of performance or sib testing pigs which need to be excluded from breeding stock.

Schmidt et al. (1971) suggested the measurement of metabolite levels in muscle tissue obtained by biopsy techniques. They observed that increased concentrations of glucose-6-phosphate (G-6-P) and lactate in biopsy samples taken several days before slaughter indicated that meat quality was likely to be inferior post mortem. Bickhardt (1971) also demonstrated that the concentrations of other metabolites in the Embden-Meyerhof pathway were increased in affected pigs. Allen et al. (1970), Bickhardt (1971), Richter et al. (1973) and Schmidt et al. (1971) observed that in susceptible pigs the activity of some enzymes in plasma was increased due to 'leakage' from muscle tissue. These enzymes included lactate dehydrogenase (EC 1.1.1.27 LDH), asparate amino-transferase (EC 2.6.11.Aspγ), alanine amino transferase (EC 2.6.1.2.AIT), malate dehydrogenase (EC 1.1.37.MDH) and creatine kinase (EC 2.7.3.2.CK). CK was the most closely correlated with stress susceptibility but subsequently several studies have presented conflicting conclusions on the value of CK for predictive purposes. Schmidt et al. (1971) demonstrated a correlation coefficient of +0.36 between plasma CK activity and meat colour (measured as % transmission), but subsequently Schmidt et al. (1974) failed to find a significant correlation between CK activity and post mortem parameters in 118 cross-bred market hogs. Addis et al. (1974) reported significant correlations between pre-slaughter CK measurements and the meat quality of 46 pigs, whereas Allen (1973, unpublished) had previously failed to demonstrate a significant correlation between the CK activity of single blood samples, collected approximately 14 days before slaughter, and the meat quality of 94 pigs of 3 breeds.

Recently the use of a relatively short exposure to halothane, which causes a characteristic rigidity in susceptible pigs, has been advocated as a suitable test procedure for use on farms (Eikelenboom & Minkema, 1974). This has serious limitations, including the risk of hepatotoxicity to the operator (Lancet, 1975).

The reasons for the variation observed in plasma CK activity have not been elucidated. They include muscular activity as suggested by Griffiths (1966) as well as gross muscle damage, and Meltzer (1971) also reported that CK activity increased during treatment with several pharmacological agents. He attributed the increase to the toxic effect of the drug or carrier on muscle.

This report describes studies of some of the reasons for variation in CK activity which were made in an attempt to increase the accuracy of prediction of SS and PSE from CK measurements.

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Materials and methods

Experiment 1

Two blood samples were collected from the ear vein of 84 pigs (Large White, Landrace or Pietrain cross breeds) within 14 days after weaning and approximately 1 week before slaughter.

In this and subsequent experiments meat quality at slaughter was recorded by EEL colour and/or transmission, as described by Schmidt et al. (1971) and the pH of the muscle M. longissimus dorsi 45 or 90 minutes after slaughter.

CK activity of plasma was measured using commercial test kits (Boehringer Corporation Ltd., 15790).

Experiment 2

To study the effects of handling, surgery and drug administration on the variability of CK activity, laboratory stock pigs and pure-bred Pietrains were surgically fitted with indwelling catheters (Tygon tubing, ID 0.065"). One end of the catheter was fixed by suture in the anterior vena cava and the other placed to emerge posterior to the ear. Blood samples were collected via the catheter, with minimum disturbance to the pig, at not greater than 24 hour intervals. The daily sample collection continued while the catheter remained patent.

To establish the effect of drugs on CK activity both azaperone, a butyro-phenone derivative and chlorpromazine, a phenothiazine derivative were administered by varying routes. Azaperone (Stresnil, Janssen Pharmaceuticals Ltd., Belgium) was administered intramuscularly at 2 mg and 4 mg/kg bodyweight and intravenously at 2 mg/kg via the indwelling catheters. Chlorpromazine was administered at 10 mg/kg intramuscularly and 5 mg/kg intravenously.

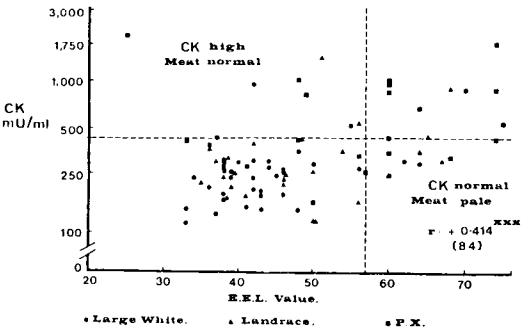
Experiment 3

Twenty Pietrain cross pigs were used to assess the effects of intramuscular injections of 4 mg azaperone/kg bodyweight on CK activity and meat quality. Blood samples were collected from the ear vein at the time of injection and 24 hours later.

Results and discussion

In the first experiment involving 84 pigs the correlation between the meat colour (EEL value) and the mean of the logarithms of the 2 CK measurements was +0.414 ($P<0.001$, see Fig. 1).

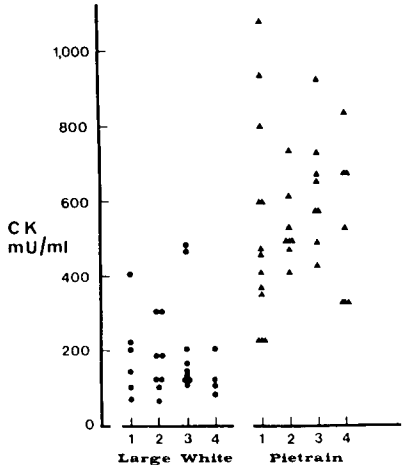
Figure 1. The relationship between meat colour and plasma CK activity.



Observations on the 39 stress resistant Large White pigs suggest that 95% of CK values will be less than 418 units and 95% of EEL values will be below 57. Using these calculated limits 5 of the 10 Pietrain cross pigs which exhibited inferior meat quality did not have abnormally high CK activity. At the other extreme 5 pigs with satisfactory meat quality had high CK activity.

Some of the factors which alter CK activity were investigated in the second series of experiments. Figure 2 shows the large day to day variation which is greater in the stress susceptible breeds. Beerman et al. (1975) have studied the reproducibility of daily CK measurements for 5 days.

Figure 2. The day to day variation in plasma CK activity in individual pigs.



They found that 64 % of variation was due to differences among animals as opposed to differences among samples for a given animal but unfortunately their population contained a low proportion of stress susceptible pigs as assessed on the basis of CK activity.

Another important factor to be considered is the duration, or apparent half life, of any increase in CK activity. In pigs which have suffered a single episode of gross muscle damage (as occurs after extensive surgery) CK activity rises during the first 24 hours and then declines during the following 48 hours. This suggests that the half time of clearance for the released CK is approximately 20 hours.

A similar duration of effect is seen following the intramuscular administration of azaperone and chlorpromazine. No increase occurred following intravenous administration and only a slight increase followed intramuscular injection of carrier solution, suggesting that the effect is due to local muscle damage by the drug itself and is not a generalised or systemic effect. Meltzer (1971) concluded that chlorpromazine exerted its effect on CK in man in a similar way.

The increase in activity appears to be exacerbated in stress-susceptible strains of pigs (Fig. 3 a & b).

Figure 3 a. The effect of injection of azaperone and chlorpromazine in 3 Large White pigs.

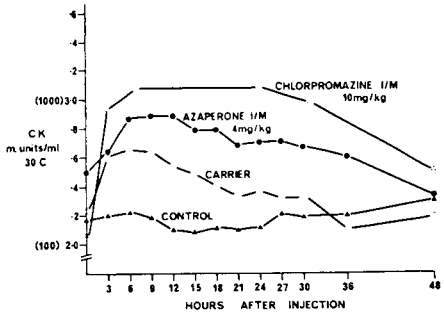
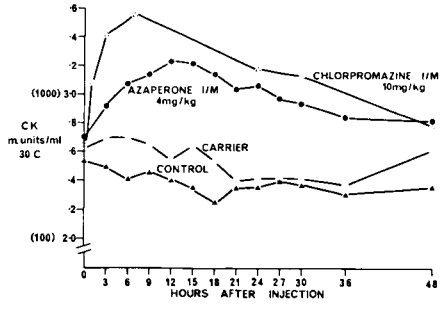


Figure 3 b. The effect of injection of azaperone and chlorpromazine in 5 Pietrain pigs.



However, an attempt in experiment 3 to use this pharmacological stress system has failed to increase accuracy in predicting stress susceptibility. In 20 Pietrain cross boars the CK activity increased from a mean value (geometric mean) of 622 to 1520 IU's within 24 hours of injection of 4 mg/kg azaperone. Neither the absolute plasma CK activity nor the induced increase in CK activity was related significantly to meat quality at slaughter.

Bickhardt (1971) demonstrated that the stress of a standard amount of physical exercise resulted in increases in CK similar to those which we have induced pharmacologically. The response 24 hours after exercise was greater in the stress susceptible pigs, but there appeared to be as wide a distribution of enzyme activities as in our experiments.

Thus many factors may affect plasma CK activity and until they can be defined and controlled its measurement to predict stress susceptibility cannot be advocated as a practical test. Systematic study of the physio-pathological factors controlling CK activity and other variables in blood is however probably still the most promising method for improving the prediction of stress susceptibility.

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THE APPLICATION OF THE HALOTHANE-TEST. DIFFERENCES IN PRODUCTION CHARACTERISTICS BETWEEN PIGS QUALIFIED AS REACTORS (MHS-SUSCEPTIBLE) AND NON-REACTORS

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Summary

Two experiments are reviewed, in which a total of 690 young Dutch Landrace pigs were subjected to inhalation of halothane and qualified as reactors (MHS-susceptible) or non-reactors (non-susceptible). The procedure of this so-called halothane-test is described. In these experiments it was found that reactors, when compared with their non-reacting control animals, had:

- elevated serum creatin kinase and aldolase levels
- a slower growth rate under ad libitum but not under restricted feeding conditions
- a lower back fat thickness and carcass length and higher ham, shoulder and total meat percentage as well as meat bone ratios in ham and shoulder
- higher death losses during the growing and finishing period
- at 45 min. after normal slaughter lower muscle pH and higher muscle temperature and rigor scores and at 24 hrs post mortem inferior muscle protein solubility and subjective evaluation scores. Although no effect of sex on the susceptibility to MHS, as tested with halothane, was found, sarcoplasmic proteins in barrows seem to be less sensitive to PSE-causing factors.

In a one year survey at the testing station, a total of 1304 Dutch Yorkshire and 1640 Dutch Landrace pigs were tested with halothane. Some preliminary results of this field experiment are reported which are in agreement with the previous experiments.

It is suggested that application of the halothane-test in a selection and breeding programme directed to optimal meat quality and stress-resistance would be most successful.

Introduction

Previous studies carried out in this laboratory have shown that stress-susceptible pigs, i.e. pigs which cannot tolerate ante mortem stress conditions or which show a high incidence of pale, soft and exudative (PSE) muscle upon normal slaughter, are hypersensitive to halothane (Fluothane, ICI) anaesthesia. The symptoms they develop when subjected to this type of anaesthesia closely resemble those which may occur in these pigs during conditions of physiological stress or exercise. The condition, characterized by progressive hyperthermia, severe muscle rigidity and metabolic acidosis, has been defined as Malignant Hyperthermia Syndrome (MHS)(Eikelenboom and Sybesma, 1969; Sybesma and Eikelenboom, 1969; Allen et al., 1970).

Our results show that in young pigs the syndrome provoked by halothane need not be lethal provided that appropriate measures are taken immediately it starts to develop (Eikelenboom and Minkema, 1974).

Research has recently been focussed upon the development of various parameters for stress-susceptibility and abnormal muscle quality, measured in the live animal, which offer a way of selecting against these traits in commercial pig breeding (Cassens et al., 1975).

This paper describes the application of the so-called halothane-test in young pigs and reviews the work we have done into the relationship between the reaction of the animal to the test and stress-susceptibility, PSE and other production characteristics.

The halothane-test

The mobile anaesthesia-apparatus (Loosco, Amsterdam) currently used in this laboratory, consists of an oxygen cylinder (10 L) with trolley, a safety regulator with rotormeter (calibaration 0 - 15 L oxygen/minute), a Fluotec Mark 3 - vaporizer (Cyprane Limited, Keighly, U.K.) and a Magill semi-closed tubing system. On the day of testing no feed is given to the animals prior to testing.

The test is conducted by two people who force the young pig (7 - 12 weeks of age) to inhale a mixture of oxygen ($2\frac{1}{2}$ liter per minute) and 5 % halothane through a face-mask, carefully observing its reaction.

Usually the pig becomes relaxed and unconscious within one minute and can be laid down on a table. A typical positive reaction is the progressive development of tonic muscular spasm and rigidity with extreme extension of the hind legs. However, sometimes the front legs and back musculature are also involved. The tonic phase of muscular spasm is sometimes preceded by clonic spasm and restlessness. The time lapse between the start of the treatment and the observation of the first symptoms of MHS (usually 2 -3 minutes in Dutch Landrace pigs), as well as the progressiveness of these symptoms, may vary.

As soon as the first symptoms of a progressive muscular rigidity are observed, treatment is immediately stopped and the animal designated as a reactor. In non-reacting animals the duration of the treatment is five minutes, which is prolonged if there is any

doubt about the animal's reaction to halothane.

Experiment 1

In the first experiment (Eikelenboom and Minkema, 1974) a total of 231 Dutch Landrace barrows and gilts with an average age of 15 weeks were subjected to the halothane-test. Thirty pigs (13 %) showed signs of MHS. None of the pigs tested died from this treatment. Since rectal temperature during anaesthesia did not differ significantly between reacting and non-reacting pigs, treatment was apparently stopped in time, preventing the development of hyperthermia in reacting pigs. The proportion of reacting gilts and barrows was not significantly different.

Reactors and non-reactors were housed randomly in groups of 4 pigs and fed "to appetite" twice a day with a normal commercial diet.

Activities of CPK and aldolase, but not GOT, in serum taken a week before slaughter was higher in reactors than in non-reacting control animals (table 1).

One of the reactors and none of the non-reactors died during the transport to the slaughter-house. Ham and loin muscle of reactors had significantly lower pH and higher temperatures at 45 minutes post mortem, while rigor mortis also occurred more rapidly in these carcasses (table 2). However, in the sows but not in the barrows, protein solubility (Percentage transmission; 't Hart, 1961) and subjective meat quality score at 24 hrs post mortem were found to be significantly different between reacting and non-reacting animals. These observations show that the (sarcoplasmic) proteins are apparently less sensitive to denaturating factors in barrows than in gilts.

Daily gain (g) during the fattening period was significantly lower in reacting gilts compared with non-reacting gilts (523 $\pm$ 73 vs 603 $\pm$ 72). A similar tendency was found in the barrows (635 $\pm$ 80 vs 661 $\pm$ 68).

Evidence was found that reactors were leaner and meatier than non-reactors since dressing percentage was higher and carcass length and back fat were lower in these animals. However, these relationships were more thoroughly investigated in the second experiment.

Experiment 2

In this experiment (Van Eldik, 1975) a total of 238 Dutch Landrace boars and 221 Dutch Landrace sows were selected from 87 litters and each litter, once they had reached a live weight of 25 kg, were paired for treatment groups: ad libitum versus restricted feeding. Boars were housed and fed individually, sows in groups of 2 to 8 per pen.

Two to three weeks after the beginning of the experiment, all the animals were subjected to halothane. The average percentage of reactors was 19.39, with no significant difference between boars and sows. Three out of 459 animals (0.65 %) died as a direct consequence of the test, all being reactors. During the growing and finishing period, including transport to the slaughter-house, losses in reactors were more than three times greater than in non-reactors (6.76 % vs 1.89 %).

No significant differences were found in daily gain, feed conversion (only boars) or ultrasonic back fat between reactors and non-reactors with restricted feeding (table 3). However, under ad lib. conditions the daily gain was lower in reacting boars and sows than in the non-reacting control animals. The figure obtained for the boars indicated that this was due to a lower feed intake in these animals as compared with the non-reacting control animals.

Carcass composition data were collected from approximately half the number of boars (table 4). Increased ham, shoulder and total meat percentages were found in reactors under both feeding levels. This increased meat percentage was apparently not only due to a decreased fat percentage but also to an increased muscle to bone ratio in reacting animals, as could be concluded from the figures presented for the dissected ham and shoulders. These data suggest therefore that the reacting animals were of a leaner and meatier type.

Experiment 3

On the basis of the previous studies, involving pigs on the experimental farm attached to the institute, it was decided to evaluate the use of the halothane-test in a large scale field experiment.

Therefore, starting in June 1975 and for a period of one year, all litters (each consisting of 2 boars and 2 sows) sent to one testing station, were subjected to the halothane-test within 4 weeks of their arrival. The preliminary results are presented here.

A total of 2.944 pigs - 326 Dutch Yorkshire litters and 410 Dutch Landrace litters - were tested. The average percentage of reactors in the Yorkshire breed was relatively low (3.07 %). However, in the Dutch Landrace breed the percentage reactors in boars and sows was 21.6 and 22.8 %, respectively. Only two Dutch Landrace pigs died as a consequence of the test.

Since this experiment is still in progress, the data presented here are on only part of the experimental material and, therefore, the results should also be considered as being very preliminary.

The growth of Dutch Landrace boars and sows does not appear to be significantly different between reacting and non-reacting animals but the thickness of the ultrasonic back fat, measured only in the boars, is significantly lower in reacting animals (table 5).

Percentage death losses at the testing

station during the period of the performance test and prior to delivery were in the Dutch Landrace breed in reactors and in non-reactors 1.72 and 0.22 %, respectively. Death losses during transport of D.L.-sows to the slaughter-house were 4.76 % in reacting and 0.46 % in non-reacting animals.

In table 6 preliminary carcass and meat quality data collected from the D.L.-sows are summarized. Differences found between reactors and non-reactors agree very well with the observations made in previous experiments.

Discussion

Our results indicate that susceptibility to the malignant hyperthermia syndrome, as indicated in young pigs by their reaction to halothane, is a good predictor of production characteristics in Dutch Landrace pigs.

To summarize, the experiments reported here show that reactors possess a superior carcass composition and an inferior meat quality, while losses due to stress are higher in these animals. This inverse relationship between meat quality and quantity seems to be even stronger than has been previously assumed on the basis of post mortem meat quality criteria. A possible explanation for this might be the occurrence in these previous experiments of phenomena as registered in the first experiment, in which a disagreement between quality parameters measured at 45 min. and 25 hrs post mortem was found in reacting barrows. In addition, environmental factors associated with stunning and slaughter also have an effect on the ultimate quality of the meat.

The results obtained so far with the halothane-test in the Dutch Landrace breed are better than those obtained with serum enzyme or muscle biopsy analysis as predictors of stress-susceptibility and meat quality. The test is also relatively cheap, easy to perform and to interpret and can easily be incorporated in a performance testing system. We have also presented strong evidence that the inheritence of MHS-susceptibility, as indicated by halothane, might be based on a single recessive gene (Minkema et al., 1976).

It is therefore suggested that the application of the halothane-test in a selection and breeding programme directed to optimal meat quality and stress-resistance would be most successful.

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Table 1. Enzyme activities in serum taken a week before slaughter from Dutch Landrace pigs, reacting (+) or non-reacting (-) with symptoms of the MHS when subjected to halothane (Experiment 1).

	+	-
n	25	25
CPK	591 ± 578	395 ± 591 [1)] [2)]
aldolase	10.2 ± 2.7	7.1 ± 3.8 [2)]
GOT	15.0 ± 5.9	14.9 ± 7.0

[1)] $P < 0.05$ } Wilcoxon
[2)] $P < 0.01$ }

Table 2. Meat quality traits in Dutch Landrace gilts and barrows, reacting (+) or non-reacting (-) with symptoms of the MHS when subjected to halothane (Experiment 1).

	Gilts		Barrows	
Traits	+	-	+	-
n	15	90 [3)]	14	111 [3)]
45 Min.post mortem				
pH (M.Semimem.)	5.93 ± 0.29	6.32 ± 0.29 [3)]	5.87 ± 0.28	6.41 ± 0.28 [3)]
(M.Long.)	5.86 ± 0.22	6.34 ± 0.37 [3)]	5.88 ± 0.36	6.40 ± 0.29 [3)]
Temperature (°C)				
(M.Semimem)	40.82 ± 0.59	40.06 ± 0.65 [1)]	40.79 ± 0.51	39.70 ± 0.74 [3)]
(M.Long.)	41.00 ± 0.82	40.42 ± 0.72 [3)]	41.28 ± 0.66	40.25 ± 0.77 [3)]
Rigor score	7.07 ± 2.59	4.13 ± 2.32 [3)]	6.50 ± 1.70	2.79 ± 2.21 [3)]
24 h post mortem				
Transmission %	73.1 ± 19.2	44.7 ± 25.1 [3)]	46.3 ± 26.6	37.8 ± 22.0
Subj.qual.score	3.43 ± 0.76	2.34 ± 0.90 [3)]	2.29 ± 0.83	2.13 ± 0.92

[1)] $P < 0.05$ [3)] $P < 0.001$

Table 3. Growth traits of Dutch Landrace boars and gilts reacting (+) or non-reacting (-) upon being subjected to halothane, in relation to feeding level (Experiment 2).

	Restricted feeding		Ad libitum feeding	
	+	-	+	-
Number of boars (individual feeding)	21	97	26	92
Weight at slaughter (kg)	102.3	102.6	103.3	103.9
Daily gain	668	667	797	842 [1)]
Daily feed intake (kg)	1.80	1.79	2.21	2.37 [1)]
Feed conversion rate	2.71	2.69	2.78	2.82
Ultrasonic backfat (mm)	17.7	18.4	17.8	19.4
Number of gilts (group feeding)	18	86	16	89 [1)]
Weight at slaughter (kg)	94.6	92.4	88.3	94.7
Daily gain (gr)	595	588	674	722
Ultrasonic backfat (mm)	15.7	15.8	16.8	17.5

[1)] $P < 0.05$

Table 4. Carcass and meat quality characteristics of Dutch Landrace boars reacting (+) or non-reacting (-) upon being subjected to halothane, in relation to feeding level (Experiment 2).

	Restricted feeding		Ad libitum feeding	
	+	-	+	-
Number of boars	9	46	14	44
Av. backfat thickness (mm)	25.9	26.2	25.8	27.8 [2)]
Ham %	22.10	21.09	21.70	20.73 [2)]
Loin %	18.96	18.53	18.83	18.46 [2)]
Total meat %	55.20	53.27	54.86	52.57 [2)]
Meat: bone ratio ham	8.36	7.57 [2)]	8.17	7.60 [2)]
Meat: bone ratio schoulder	5.37	4.87 [3)]	5.25	4.82 [2)]
pH_1 semimembranosus	6.07	6.63 [3)]	6.14	6.56 [2)]
pH_1 longissimus	5.89	6.45 [2)]	6.14	6.38 [3)]
Rigor score	4.22	1.72 [2)]	5.86	2.89 [3)]
Meat quality score [*)]	2.11	3.37 [2)]	2.50	3.30 [3)]

[*)] subjective evalution: 1: serious PSE; 2: slight PSE; 3: normal; 4: good

[2)] $P < 0.01$ [3)] $P < 0.001$

Table 5. Growth traits of Dutch Landrace boars and sows reacting (+) or non-reacting (-) upon being subjected to halothane (Experiment 3, preliminary results).

	Boars		Sows	
	+	-	+	-
Number	111	450	117	440
Weight at slaughter (kg)	98.05	98.01	98.90	98.55
Daily gain (gr)	809	815	764	759
Daily feed intake (kg)	2.095	2.104	2.140	2.143
Feed conversion rate	2.595	2.586 [3)]	2.811	2.831
Ultrasonic backfat (mm)	11.44	12.19 [3)]	-	-

[3)] $P < 0.001$

Table 6. Carcass- and meat quality traits in reacting and non-reacting Dutch Landrace sows (Experiment 3, preliminary results).

	Reactors	Non-reactors
Number sows	105	437 [2)]
Slaughter loss %	22.42	22.91 [3)]
Carcass length (cm)	83.40	84.03 [3)]
Av. backfat thickness (mm)	22.58	23.86 [3)]
Loin %	20.17	19.76 [3)]
Ham %	27.70	26.80 [3)]
Meat Quality [*)]	5.55	6.70 [3)]
Transmission	50.37	31.44 [3)]

[2)] $P < 0.01$ [3)] $P < 0.001$

[*)] meat quality score, scale 4 (serious PSE) - 8 (good)

EFFECT OF SUSCEPTIBILITY TO THE MALIGNANT HYPERTHERMIA SYNDROME (MHS) AS DETECTED BY HALOTHANE ON SOME PRODUCTION AND ENERGY BALANCE CHARACTERISTICS IN DUTCH LANDRACE PIGS

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Summary

Various parameters in MHS-susceptible (pos.) and non-susceptible (neg.) animals (detected by Halothane) were measured in different experiments.
1. Pos. and neg. animals (12 in each group) were individually fed at 93 g of food/kg$^{0.75}$ and housed in groups of four (2 pos. and 2 neg.) from 30 to 100 kg. No differences in fattening traits were detected. Meat quality was much less in pos. animals as measured by pH, rigor and transmission value. Pos.animals were shorter and had higher meat % in the carcass (56.3% vs 53.9% resp.).
2. Respiratory heat exchange was determined in 4 experiments at a feeding level of 93 g/kg$^{0.75}$. In each experiment 2 groups of 9 pigs were subjected to temperatures, rising from 20 to 28°C during week 1, of 30°C during week 2 and decreasing from 28 to 20°C in week 3, in the weight range 25-30 kg and also in the weight range 85-100 kg. No differences in respiration coefficients and in heat production over 48 h periods were detected between pos. and neg. animals.
3. N balances were determined in 4 pos. and 4 neg. animals at about 30 kg and at 85 kg at a feeding level of 93 g/kg$^{0.75}$. No differences between pos. and neg. animals were found.
4. Feed intake was determined in 10 pos. and 10 neg. animals fed ad libitum and housed individually. Pos. animals showed a significant lower feed intake and a lower body weight gain over a 3 week period in the weight range 50 to 100 kg (about 5% less feed intake).

The results of these experiments show that fattening traits in pos. and neg. animals did not differ when fed at the same restricted feeding level, but body length is reduced and meat quality is much less while meat % was increased in pos. animals. Respiration coefficients and energy and N balances did not differ between pos. and neg. animals when housed at 20 to 30°C and fed at the same feeding level. When fed ad libitum pos. animals had a lower feed intake and a corresponding lower growth rate.

Introduction

Susceptibility of fattening pigs to Porcine Malignant Hyperthermia Syndrome (MHS) causes an important loss in production of meat by death before slaughtering and also by the loss at retail due to decreased meat quality (Cassens, Marple and Eikelenboom, 1975). Eikelenboom and Minkema (1974) and Van Eldik (1975 a,b) have found that MHS-susceptibility also influenced some fattening traits. Animals which are MHS-susceptible (pos. reactor as detected by Halothane) showed reduced weight gain at semi-ad libitum and ad libitum feed intake compared to non MHS-susceptible animals (neg.=non-reactor). The pos. animals had decreased carcass length and increased meat %.
Kallweit and Haase (1971) suggested that stress susceptible pigs may have a higher metabolic rate at rest than non-susceptible pigs. In a number of experiments with pos. and neg. Dutch Landrace pigs various parameters which may cause reduced body weight gain and/or increased meat % were investigated
- Rate of gain and carcass quality at group housing and individual feeding
- Respiratory heat exchange and energy balances in groups of pos. and in groups of negative animals at constant feeding level at moderate and high temperatures
- N balances at constant feeding level
- Feed intake and rate of gain at ad libitum feeding.

Material and Methods

Animals

In all four experiments Dutch Landrace pigs were used, reared and tested for their susceptibility to Halothane at the experimental farm of the Research Institute for Animal Husbandry in Zeist.
In Table 1 initial and final weights of pigs in each experiment (or stage of experiments), duration of experiments and housing and feeding characteristics are presented.
1. In experiment 1, 12 pos. and 12 neg. pigs (8 females and 4 castrated males in each group) were used. They weighted 20-40 kg (mean about 35 kg) at arrival and were fed to 95-100 kg.
2. Two groups of pos. and two groups of neg. pigs (8 females and 8 castrated males in each group) were put in the calorimeter of the department (Verstegen and Van der Hel, 1974) when weighing 24-30 kg and kept there for 24 days. At 75-85 kg 10 animals out of the 16 (5 females and 5 castrated males)

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were again put in the calorimeter for 24 days.

3. The castrated males from experiment 1 were used in N balance trials at about 28 kg and again at 74 kg.
4. In experiment 4, 10 pos. and 10 neg. animals were used. Each group of animals consisted of 6 castrated males and 4 females. Each pair of a pos. and a neg. animal of the same sex originated from the same litter. The pigs weighed between 39 and 90 kg at arrival and had been restrictedly fed before use in this experiment.

Feeding

In all experiments food with a constant content of 12.0 kJ metabolizable energy (ME) per gram was used. It contained about 2100 kcal NEFs and 13.2% digestible crude protein (Cöp, 1974). The food had the same composition as normally used in testing stations. When not fed ad libitum the animals received 93 g/kg$^{0.75}$ per day based on weekly predicted body weight and they received the food twice daily as wet mash.

Treatment

Experiment 1

The 12 pos. and 12 neg. animals used in this experiment were housed in groups of 4 and fed individually. The animals were housed in a temperature controlled pig house (mean temp. 16.5°C). The groups of 4 pigs were composed of 2 pos. and 2 neg. animals of about the same weight in one pen. The animals were weighted once a week and food was given to them on the basis of predicted weight in this weekly periods. After slaughtering carcass quality and meat quality was determined as described by Bergström and Kroeske (1968) and by Eikelenboom and Minkema (1974).

Experiment 2

Each group of 16 animals was put in the calorimeter when weighing 22-25 kg. In the calorimeter 8 females and 8 castrated males were separated and each subgroup of 8 placed in a pen. The animals were housed on asphalt and group fed. After a preliminary period of 3-4 days at 20°C the pigs were subjected to temperature treatments during 3 weeks. The first week the temperature was increased in stepwise fashion every 2 days with 2-3°C from 20 to 27.5°C. In the 2nd week the temperature was kept constant at 30 C and in the third week the temperature decreased in stepwise fashion with 2-3°C every 2 days from 27 to 20°C. Respiratory gaseous exchange was measured 3 times 48 hours each week. The relative humidity was kept at 65-75%. At about 85 kg body weight this procedure was repeated.

Experiment 3

When weighing about 28 kg 4 pos. and 4 neg. castrated males were placed in metabolism cages and provided with a harness for collection of feaces. After a preliminary period of 4 days N balances were determined from N in food, N in feaces and N in urine for two successive periods of one week each. After the collection period which lasted 7 days the animals were treated similar to the females in exp. 1. The procedure was repeated when the pigs weighed about 75 kg.

Experiment 4

In this experiment 10 pos. and 10 neg. pigs were housed in the temperature controlled pig house. Each pig was housed individually in a pen and had about 12 m^2 aerea for lying and dunging. The animals had wood shavings as bedding. Feed intake and feed intake behaviour was determined continuously during 3 weeks (Salden and Sas, 1976). For the purpose of the present study feed intake was determined 3 times each week during the four weeks duration of the experiment (and 3 weeks for pair 9 and 10).

Methods of analyses

In experiment 1 there was a large variation between mean weights of animals. Therefore individual growth curves between 30 and 80 kg have been smoothed and derived therefrom were ages at certain weights, weights at certain ages and cumulative feed intake at certain weight and age. The data on gain, feed intake, ages and carcass quality and meat quality characteristics have been analysed according to the model

$$Y_{ijk} = \mu + h_i + s_j + p_{h:j} + e_{ijk} \quad (1)$$

in which

Y_{ijk} = parameter
h_i = stress susceptibility(i=1,2)
s_j = sex (j=1,2)
$p_{h:j}$ = pen
e_{ijk} = error

In experiment 2 the data on energy balance traits have been analysed according to

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_l + ab_{ij} + ac_{ik} + ad_{il} + bc_{jk} + bd_{jl} + cd_{kl} + e_{ijklm} \quad (2)$$

in which

Y_{ijklm} = trait
a_i = stress susceptibility(i=1,2)
b_j = weight class (j=1,2)
c_k = temperature treatment (k=1,3)

d_l = replicate (l=1,2)

$ab_{ij} + \ldots + cd_{kl}$ = interactions

e_{ijklm} = error

In experiment 3 and 4 the data from the N balance trial and feed intake have been tested by the F test. The data on food intake in exp. 4 have been corrected towards the mean weight of each pair of 1 pos. and 1 neg. littermate by the allometric equation

$$\hat{y}_i = y_i + a\,(G + \Delta G)^p - aG^p \qquad (3)$$

in which

$\hat{y}_i$ = corrected food intake

y_i = observed food intake

G = mean body weight of pair of animals

ΔG = deviation of mean weight of a pig from the mean weight of its pair

a and p = coefficient and exponent calculated per pig

Results

Experiment 1

In Table 2 the performance traits and data on carcass quality and meat quality have been presented. Differences in fattening traits were not significant. Meat quality was significantly inferior in MHS-susceptible animals. Meat % was increased and carcass length and backfat thickness were significantly lower in pos. animals.

Experiment 2

Energy balance (EB) was calculated as difference between ME intake and heat production and the maintenance requirement (MEm was obtained by subtracting ME for production MEp) from total ME intake. MEp was obtained by EB/0.7, thus assuming a partial efficiency of 0.7 for conversion of MEp into EB. In Table 3 the energy balance data have been given as least square means in pos. and neg. animals for the low and high weight class (app. 30 and about 85 kg respectively) in the successive weeks of the temperature treatments. Differences in energy metabolism traits between pos. and neg. animals were not significant. Heat production at about 85 kg was lower than at about 30 kg ($P < 0.01$). Maintenance requirement was accordingly reduced and energy balances increased. At higher body weight the heat production in the week at 30°C was increased but not in the low weight range, which was reflected in a pos. interaction of weight class temperature treatment ($P < 0.05$). Respiration coefficients in pos. and neg. animals did not differ significantly (mean 1.11 vs 1.12).

Experiment 3

The data on N balances and food intake are given in Table 4. Differences in traits between pos. and neg. animals with respect to N balances are very small and not significant.

Experiment 4

In Table 5 the results of the pos. and neg. littermates on food intake and weight gain have been given. One pair (5) had to be excluded due to illness of one animal. It is clear that in all pairs (except pair no. 1 and 9) the weight of the pos. littermate is less than in the neg. littermate. Therefore food intake was corrected towards mean pair weight. After correction the food intake in pos. animals is less than in neg. animals. This difference within littermates is significant ($P < 0.01$). Also body weight gain is lower in pos. animals.

Discussion

The presence of susceptibility to Porcine MHS in pigs may have important consequences for pig production. From the investigation of Eikelenboom and Minkema (1974) there is evidence that fattening traits can be influenced by MHS susceptibility. The present study was carried out to investigate possible causes of this difference in performance. First of all daily gain and food conversion was followed in pos. and neg. animals when housed together but fed individually at the same feeding level. Due to the difference in weight at start the smoothing technique was chosen to calculate gain and food conversion at certain weights and ages in the interval 30-90 kg. No significant differences in these traits between pos. and neg. animals were detected. However carcass length was reduced and meat % was increased and meat quality characteristics were inferior in MHS-susceptible animals, which agrees with the findings of Eikelenboom and Minkema (1974). Kallweit and Haase (1971) have suggested that a difference in metabolic rate between stress-susceptible and non-susceptible pigs may exist. The results of the present study do not show any effect of MHS susceptibility on energy balance traits, not even at conditions of high temperature with animals at higher body weight. However application of more stress-conditions than those used in this experiment might create possible differences in metabolic rate. The differences in heat production between animals of about 30 kg and 85 kg were as could be expected. Heavier animals showed increased heat loss at 30°C, thus it can be concluded that they were above their zone of thermoneutrality.

Both pos. and neg. animals reacted in the same way at high body weight and high temperature. Perhaps temperatures much higher than the 30°C in this investigation may create differences. One can also argue that the reduction in weight gain in the data of

Eikelenboom and Minkema (1974) may have been due to differences in the ratio protein gain to fat gain. Therefore N balances were determined at 30 kg and again at about 80 kg. With the technique employed (total collection method and Kjeldahl analyses) no differences could be found in respect to MHS susceptibility. The N balances found here are in accordance with those mentioned by various authors (see Cöp, 1974). A fourth possibility of difference in pos. and neg. animals may be the occurrence of differences in food intake at ad libitum feeding level and/or in restricted feeding when pos. animals have to compete with neg. animals at the trough.

Only the ad libitum feeding level in individually housed animals was studied in pos. and neg. littermates. In pos. pigs food intake was reduced by about 5%. This reduction was associated with lower rate of gain. Therefore it can be expected that differences in rate of gain between pos. and neg. animals may be attributed to differences in food intake. However difference in meat %, backfat thickness and body length simply cannot be explained from differences in food intake between both groups of pigs.

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Table 1. Number of animals, housing conditions individual (I) or in groups (G), feeding conditions (individual/group) initial and final weights and duration of experiments with MHS-susceptible (pos.) and non-susceptible (neg.) pigs.

Exp.	MHS pos/neg	Feeding level g/kg$^{0.75}$	Housing I/G	Feeding I/G	Number of animal per group/total		Mean initial weight(kg)	Mean final weight(kg)	duration of experiment (days)
I	pos	93	G	I	4	12	24.1	103.7	119
	neg	93	G	I	4	12	24.5	101.0	119
II a	pos	93	G	G	8	16	24.7	34.4	24
	pos	93	G	G	8	16	29.4	40.2	24
b	neg	93	G	G	8	16	23.4	32.9	24
	neg	93	G	G	8	16	27.2	38.5	24
c	pos	93	G	G	5	10	81.5	101.9	24
	pos	93	G	G	5	10	87.4	108.9	24
d	neg	93	G	G	5	10	77.8	94.6	24
	neg	93	G	G	5	10	85.8	103.3	24
III a	pos	93	I	I	1	4	28.5	37.5	14
	pos	93	I	I	1	4	73.9	84.4	14
b	neg	93	I	I	1	4	28.6	36.6	14
	neg	93	I	I	1	4	74.6	84.9	14
IV	pos	ad lib.	I	I	1	10	68.4	95.9	28/21
	neg	ad lib.	I	I	1	10	73.4	103.9	28/21

Table 2. Fattening traits, carcass quality and meat quality of MHS-susceptible (pos.) and non-susceptible (neg.) Dutch Landrace pigs (± SE). Experiment 1.

MHS susceptibility	Pos.	Neg.	Significance of difference
No. of animals	12	12	
Initial weight (kg)	24.1	24.5	NS
Final weight (kg)	102.8(1.5)	100.4(1.5)	NS
Daily gain[1] g/d	728	709	NS
Food conversion ratio[1]	2.95	3.01	NS
Carcass weight (kg)	79.2(1.4)	76.7(1.4)	NS
100-slaughter loss (%)	76.9(0.5)	76.3(0.5)	NS
Carcass length (mm)	820(8)	849(8)	**
Carcass quality (scale 1-5)	2.12(1.99)	2.75(1.99)	**
Pr. backfat thickness (mm)	28.4(0.8)	28.9(0.8)	*
Meat %	56.3(0.7)	53.9(0.7)	**
Leannes (scale 4-9)	8.0(0.1)	7.8(0.1)	NS
Firmness (scale 4-8)	4.8(0.3)	5.9(0.3)	**
Color (scale 4-8)	4.8(0.4)	5.9(0.4)	**
pH (l.d.)	6.0(0.1)	6.5(0.1)	***
Rigor score	6.8(0.8)	2.9(0.8)	***
Transmission value units	78.0(7.7)	49.6(7.7)	**

1. up to 90 kg * P < 0.10 ** P < 0.05 *** P < 0.01

Table 3. Heat production (HP), metabolizable energy intake (ME), energy balance (EB) maintenance requirement expressed as $kJ/kg^{0.75}$ per 24 hrs in MHS-susceptible and non-susceptible Dutch Landrace pigs and weight in various classes. Least square means (± SE) per subclass. Experiment 2.

Subclasses	weight (kg)	Heat Production $kJ/kg^{0.75}$	Metabolizable Energy $kJ/kg^{0.75}$	Energy Balance $kJ/kg^{0.75}$	Maintenance MEm in $kJ/kg^{0.75}$	Number of measurements
pos.	61.3(0.3)	654.1(3.5)	1115.7(1.8)	461.6(4.0)	456.4(5.1)	38
neg.	64.4(0.3)	650.4(3.5)	1108.8(1.8)	458.3(4.0)	454.4(5.8)	37
low weight	31.8(0.3)	[661.6(3.5)] ***	[1114.8(1.8)] ***	[453.2(4.0)] ***	[467.4(5.1)] **	38
high weight	93.9(0.3)	[642.9(3.6)]	[1109.7(1.8)]	[466.7(4.0)]	[443.0(5.2)]	37
week 1(20-27°C)	57.7(0.3)	648.4(4.0)	1111.6(2.0)	463.2(4.5)	449.9(5.8)	29
week 2 (30°C)	63.4(0.4)	657.2(4.4)	1108.0(2.2)	450.7(5.0)	464.1(6.3)	24
week 3(27-20°C)	67.4(0.4)	651.3(4.6)	1117.2(2.3)	465.9(5.2)	456.7(6.6)	22

differences between classes [ ] ** P < 0.05 *** P < 0.01

Table 4. Food intake and N balances and mean weight in MHS-susceptible and non-susceptible Dutch Landrace pigs. Experiment 3.

MHS susceptibility	Pos.		Neg.	
Number of animals	4	4	4	4
Mean weight (kg)	33.0	79.1	32.6	79.8
Food g/d	1248	2459	1232	2461
N balance g/d	13.86	17.57	13.62	17.50

Table 5. Food intake and body weight gain in MHS-susceptible and non-susceptible littermates. (Experiment 4). Each pair selected from one litter.

	MHS-susceptible			Non-susceptible		
Pair no.	Food intake g/d	gain g/d	weight kg	Food intake g/d	gain g/d	weight kg
1	4882	1191	105.6	4380	1048	104.0
2	3343	1098	86.3	3540	1036	96.5
3	3226	982	71.4	3886	1232	78.5
4	3851	1036	92.3	4621	1339	106.6
6	3451	857	79.1	4682	1250	85.8
7	2220	857	48.8	2894	964	55.4
8	3717	946	98.5	4214	1179	107.1
9	3975	1304	75.1	3706	1196	74.9
10	4228	1214	80.0	4921	1464	86.6
Mean	3655	1053	81.9	4094	1190	88.4
Mean after[1] correction:	3681			4060		

1. Food intake corrected to mean body weight in each pair of littermates.

THE VALUE OF VARIOUS MEAT QUALITY CHARACTERISTICS IN ESTIMATING BREED DIFFERENCES IN PSE-SUSCEPTIBILITY

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Summary

Norwegian Landrace was used as a common control stock in an international co-operative experiment in order to compare the genetic merit of landrace breeds. In each of the seven participating countries the native landrace breed was compared with progeny of this control stock. As a part of the experiment in The Netherlands various meat quality characteristics were measured in five different breeds, viz. Norwegian Landrace, Dutch Landrace, Dutch Yorkshire, Piétrain and Belgian Landrace. The characteristics measured were: pH_1, ultimate pH, rigor development, transmission value, Fahellpho value, percentage drip, overall subjective scores and subjective scores for colour, firmness, wetness, texture and leanness. In addition two weeks ante mortem muscle biopsies were taken and assayed for glucose-6-phosphate and lactate. Significant breed differences were found for most of the criteria. The two breeds with the highest meat percentage, Piétrain and Belgian Landrace, had the worst meat quality. In spite of this the correlation coefficients between meat percentage and meat quality characteristics, though sometimes significant, appeared to be small.
An attempt was made to find a small subset of the variables measured that would be sufficient to describe breed differences with respect to meat quality, by repeated testing for additional information contained in the variables not included. Many variables were needed for description of breed differences, however. A component analysis revealed that the ultimate pH and the subjective score for leanness seemed to be more or less isolated from the other meat quality characteristics. The latter were subsequently handled in a factor analysis. High loadings (>.80) were found for the subjective scores and the transmission value, and somewhat lower loadings for Fahellpho value and percentage drip. This analysis suggested that if these characteristics can be described with one common factor, the subjective quality scores are of most value with in addition perhaps transmission value for prediction of this common factor. If two factors have to be considered also pH_1 should be measured in addition.

Introduction

The processes taking place in swine skeletal muscles after slaughtering which lead to meat with an aberrant quality have been studied extensively and various factors of genetical, endocrinological, physiological as well as environmental origin appear to contribute to the development of pale, soft, exudative meat (Bentler, 1972; Cassens et al., 1975). It is generally accepted that this deviation in meat quality is mainly a consequence of an enhanced glycolysis in the muscle during the early post mortem period. Therefore mainly muscle pH is measured and occasionally levels of lactate, glucose-6-phosphate (G6P) or other glycolytic intermediates. However to characterize the appearance of PSE-meat it is necessary to measure also the pale, soft and exudative aspects, which can be done in various ways, subjectively as well as objectively. In most studies, however, only few aspects of meat quality are measured and without defining meat quality. It is difficult to predict from such particular characteristic(s) the ultimate meat quality.
As a part of an international breed comparison using Norwegian Landrace as a common control stock (results of which are summarized in a general report elsewhere by King et al. (1975)), the experiment in the Netherlands comprised alongside Norwegian Landrace (NoL) and Dutch Landrace (DL) also Dutch Yorkshire (DY), Piétrain (P) and Belgian Landrace (BL). We utilized this opportunity to measure as many meat quality characteristics as possible, and tried to find the best predictor(s) for meat quality.

Material and methods

Experimental design and animals

The common control stock was selected from 23 litters of Norwegian Landrace pigs. One piglet from each of 7 littermates was allocated at random to each of the 7 co-operating countries. We imported 11 male and 12 female piglets. They were reared and after a multiplication phase 80 animals of their progeny have been compared with 112 DL, 96 DY, 96 P and 80 BL pigs. The fattening experiment was carried out in two replicates, piglets from first and second litters respectively. Herdbook inspectors purchased groups of four per litter: two

gilts and two castrates, from breeders that were listing regularly.

Housing

The animals were housed in groups of four in a former progeny testing station. The five breed groups were randomly distributed over the wings of the building. The four littermates were housed together. Hence the pen averages equal the litter averages.

Feeding

The pigs were fed to appetite twice daily. The feed mixture consisted of maize, grain, soybean oil meal and some minor components supplemented with minerals and vitamins, and contained 13.2 % digestible protein. It was given as pellets in wet form and throughout the whole fattening period from 25 to 100 kg. The animals were weighed every fortnight.

Carcass quality

After one night cooling the right carcass half was dissected according to the Institute's standard method (Bergström and Kroeske, 1968). By this method meat percentage consists of the percentage hams, cutlets, shoulders and meat scraps. Ham and shoulder are defatted. This fat from ham and shoulder together with backfat, lower jaw fat, flare fat and belly give the fat percentage. The remaining parts: head, feet and tail form the percentage offal. The percentages are based on cold carcass weight.

Meat quality

The circumstances at the testing station, during transport and at the slaughter house were as much as possible standardized. G6P and lactate levels were measured in 200 mg biopsy samples, taken from the longissimus muscle 14 days before slaughtering (Schmidt et al., 1972). The pH_1 values were measured ± 45 min. post mortem in longissimus muscle and semimembranosus muscle, as well as rigor mortis values (Sybesma, 1966) in the semimembranosus muscle. At dissection 24 h post mortem the subjective scores were established by 3 different inspectors. Person A and B used the same method (Walstra et al., 1971). The scores were estimated on the cross section of the longissimus muscle. Person B only scored for an overall impression; A and C also scored for the separate quality traits as mentioned in table 2. The scales used are also given in this table. Three chops were cut off. The most caudal one was used for measurement of protein solubility by means of the transmission value according to Hart (1962) and light-reflectance by means of Fahellpho value. The two remaining chops were each hung in a separate polyethylene bag for assessment of water loss. Drip was calcalated from the average amount of water found in these bags during three days and expressed as a percentage of the amount of lean meat. At that time the ultimate pH of these chops was also measured. Good meat quality is associated with higher values for pH_1 and for the subjective scores A and B, and with lower values for G6P, lactate, rigor, transmission, Fahellpho, drip and for subjective score C.

Statistical analysis

The pens were considered to be the experimental units. Litters were grouped to blocks with respect to replicates and wings of the building. The analysis was based on a model with additive contributions of the breeds, the blocks and the interaction between them. An analysis of variance was calculated for the individual variables. The significance of the breed differences can be judged by means of the least significant difference (LSD) at the 5 % level. This procedure was applied to the meat quality characteristics as well. However some special analyses were carried out on these variables in an attempt to describe meat quality with a small subset out of the variables measured. First multivariate analysis of variance techniques, especially repeated testing for additional information on breed differences contained in the variables not in the subset, were applied. Later on it was realized that a component analysis and a factor analysis could be more appropriate. In the case of factor analysis two models were tried:

$\underline{y}_i = \alpha_i + \beta_i x + \underline{e}_i$ and $\underline{y}_i = \alpha_i + \beta_i x + \gamma_i z + \underline{e}_i$

respectively,

in which $\underline{y}_i$ = the meat quality variable i

a_i = a constant

β_i and γ_i = factor loadings

x and z = meat quality factors

$\underline{e}_i$ = error term

Results

During the fattening experiment only in one pen with P animals severe infections occurred. Therefore the whole pen was eliminated from the calculations, so that 92 animals of this breed were involved. From about one third of the animals no biopsies were taken and overall subjective scores of inspector C were not assessed. The number of animals involved for each of the variables is given in the concerning tables. All results in the tables are listed over replicates and sexes.

Faatening performance and carcass quality

The results for the most important traits are shown in table 1. DY is the fastest growing breed with the best feed conversion ratio. The differences in growth rate between the landrace breeds were not significant. Feed conversion in DL was superior to the other two.

In P growth rate and feed conversion is poor in comparison to the other breeds which is mainly caused by its lower feed intake capacity. However P is superior in meat percentage. Meat percentage in BL also significantly differs from the remaining three breeds. These differences are not only reflected in fat percentages, but also in dressing percentages, carcass lengths and, except for P, in grading. With increasing leanness of the carcass the dressing percentage becomes higher, the carcass is shorter and grades better. For carcass length all breeds are significantly different from each other. This influences grading and backfat thickness. Striking is the low percentage IA in NoL, which is due to the poor type of the breed since the percentage I (judgement for backfat thickness) is as high as in the other breeds. Average backfat thickness (average of five places) is thinnest in P and NoL and differs significantly from the other three breeds. However considering the backfat thickness along the midline there are considerable differences in distribution between the breeds (King et al., 1975).

Meat quality differences between breeds

The differences found between the sexes and within breeds were small, and mostly, although not significantly, in favour of the castrates. In table 2 the results are given both for objective and subjective scores. The general picture is that DY clearly has the best and that P and BL clearly show the worst meat quality and in almost all cases significantly so. The differences between P and Bl are small, except for percentage drip and the amounts of G6P and lactate that are significantly in favour of BL. Some of the differences between NoL and DL, rigor and pH values, are significantly in favour of NoL. It is striking that on the other hand the percentage drip in NoL is the highest of all breeds and significantly worse than in DL, DY and also in BL. The subjective judgements for B and C are significantly in favour of DL compared to NoL. The relationship between stress-susceptibility and PSE is reflected in the number of deaths during transport (5 animals) and already during the fattening period (3 animals) in BL. For the other breeds the total number of deaths remained below 4.

The relationship between meat quality characteristics

In table 3 the phenotypic correlation coefficients are given. From the table it can be seen that the overall score A bears a good relationship with the other scores B and C, and with transmission and Fahellpho values ($r = -0.70$ to -0.76). Approximately similar results are obtained with the overall scores B and C. Transmission values have higher correlations with other characteristics than Fahellpho values. The percentage drip correlates highest with the subjective scores (r about 0.60) and reasonably with Fahellpho ($r = 0.54$). Both pH_1 values show good relationships with rigor value ($r = -0.62$ and -0.55); their mutual correlation coefficient is 0.78 which is the highest in the matrix. Both pH_1 values have about equal correlations with the other traits. The highest correlation for G6P is with overall score B ($r = -0.50$); for lactate it is with pH_1 of the semimembranosus muscle ($r = -0.42$). G6P correlates better with the other characteristics than lactate does. Tow third of the correlation coefficients were found to be below 0.50. Growth rate and meat percentage have a low to moderate relationship with the meat quality characteristics. The highest correlation coefficient for growth rate is reached with the amount of lactate ($r = -0.39$) and for meat percentage with rigor value (r also 0.39). With two third of the meat quality traits a correlation coefficient of 0.30 is not reached. The within breed correlation coefficients could not be listed here. They however do not deviate very much from the general picture as outlined in the given matrix, except for rigor value, G6P and lactate. The latter variables show rather different correlations with the other variables for different breeds, suggesting that their value for measurement of meat quality remains limited. Moreover the correlations are low to moderate.

The value of the variables as predictors of meat quality

The tests on additional information, given certain variables, showed that the greatest differences between the breeds were found for the overall subjective score B. After elimination of this variable rigor value appeared to be the next variable to give the greatest breed differences. Subsequent eliminations then led to the subjective scores leanness A, colour A overall score A, and percentage drip respectively. In all these cases and also after elimination of certain chosen combinations the test showed that still the remaining variables contained additional information on breed differences.
After that a component analysis was tried. All the variables, except leanness A and ultimate pH, showed high loadings on the first or the first two components. Therefore a factor analysis was carried out, excluding leanness A and ultimate pH, with 1 and 2 factors respectively. The results are shown in table 4. If the model with 1 common factor is applied high loadings are given by all subjective scores and transmission value, and somewhat lower also by Fahellpho value and percentage drip. Used as predictors of the common factor the overall score A, firmness A, colour A, overall score C, texture C (all subjective scores) and then transmission value (as the first objective score), in that order, give the best prediction as can be seen from the values of

$\hat{\sigma}^2/\hat{\beta}^2$ in table 4. If 2 common factors should be considered table 4 demonstrates that the high loadings in the first factor are almost in the same order. In the second factor then the highest loadings come from the pH_1 values and somehwat lower from rigor value. Combining these two factors the most promising additional variable in combination with overall score A, firmness A, overall score C, texture C and transmission value appears to be pH_1 for prediction of the common factors.

Discussion

The differences between the breeds in this experiment are very clear. In growth rate and feed conversion DY is by far superior to the other breeds. However the same applies to P when considering meat percentage, while this breed is by far inferior with respect to growth rate and feed conversion. The breeds DY, DL and NoL are about equal in carcass quality, the latter breed however is poor in grading, but better in average backfat thickness. BL has a position in between with respect to carcass quality. In growth rate and feed conversion BL is, although not significantly, inferior to DL.
Breed differences in meat quality are well-known, not only between breeds in the European pork producing countries but also in the USA (Wax et al., 1975). This experiment is another example of striking breed differences. Again DY is by far superior to the other breeds. Comparing NoL and DL it can be concluded that DL has a somewhat better meat quality when judged by subjective traits and that the reverse holds when judged by objective traits. The meat quality in BL was as poor as in P, but the lean body mass does differ. So a fatter carcass does not necessarily imply a better meat quality. This also applies if one compares the meat quality of DY with that of DL and NoL, because they have about the same lean body mass. The statement is in accordance with the calculated correlation coefficients between meat percentage and meat quality traits. They are at most 0.35 to 0.40, so that meat percentage only explains up to 15 % of the total variation in meat quality. Although not listed here the calculations within breeds show comparable results. In the literature in most cases the phenotypic correlations between meat quality traits and meat percentage or a variable derived from it are lower (Moen et al., 1970; Charpentier et al., 1971; Richter et al., 1973; Duniec et al., 1974; Ender and Pfeiffer, 1974; Martin and Fredeen, 1974; Martin et al., 1975; Wax et al., 1975). These low to moderate unfavourable correlations however necessitate incorporation of meat quality in selection programmes, because progress in leaner carcasses will still slowly lead to worsening of meat quality. The established heritability coefficients vary from 0.10 to 0.71 depending on the meat quality trait and the muscle involved, the breed and the sex as calculated by four of the just mentioned references and by Scheper (1973), Jensen (1974) and McGloughlin and McLoughlin (1975). The h^2 for meat colour varies from 0.17 to 0.39 (Moen et al., 1970; Duniec et al., 1974; Jensen, 1974). At our institute a h^2 for the overall score A in DL of about 0.30 was calculated (Walstra et al., 1971). So there are possibilities to include this parameter in a sib test or a progeny test. Besides a h^2 of 0.20 to 0.30 the Danish work (Jensen, 1974) showed that 30 to 40 % of the variation in meat colour is due to non-additive (dominance) gene effects.
We have no explanation for the strong deviation of percentage drip in NoL in comparison to the other breeds. Bendall et al. (1975) state that drip is related to rigor development and the ultimate pH in that way that an enhanced rigor mortis leads to a greater extrafibrillar space resulting in loss of water, and the ultimate pH is somewhat increased. This is in complete disagreement with our figures as shown in the tables 2 and 3, because rigor value and ultimate pH in NoL are the lowest of all five breeds. Moreover the correlation between rigor and drip is the lowest in the matrix ($r = 0.03$). This remains so if correlations per breed are considered.
The value of a certain characteristic as a predictor of meat quality is often based on the correlation with another trait which is supposed to be a good indicator of meat quality. However the traits may measure different aspects of meat quality. Therefore we tried to find out what is common in a great number of variables.
The approach with the multivariate analysis of variance techniques showed that many variables were needed for description of breed differences. This suggests that meat quality has many aspects or that the variables possibly measure 'something else' that also causes differences between the breeds. From the factor analysis however it can be concluded that if meat quality, as measured with the 16 variables considered, can be described with one common factor, the subjective scores, with in addition perhaps transmission value, appear to be of most value for prediction of the common factor. In addition one or more specific factors may be involved. If two common factors have to be considered also pH_1 has to be mentioned.

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Table 1. Results of fattening performance and carcass quality.

	NoL	DL	GY	P	BL	LSD
Number of animals	80	112	96	92	80	
Av. daily gain (g)	718	729	763	614	708	23
Feed conversion (kg feed/kg gain)	3.38	3.25	3.07	3.47	3.33	0.10
Feed intake (kg/day)	2.43	2.37	2.34	2.13	2.35	0.07
Dressing percentage	77.6	77.9	78.6	81.5	80.7	0.6
Classification score (IA %)	50.0	78.4	81.2	90.5	96.3	15.1
Carcass length (cm)	86.5	85.6	81.7	76.8	80.3	0.8
Av. backfat thickness (mm)	26.9	28.2	28.3	26.8	28.2	1.6
Meat percentage	54.35	54.58	54.67	59.51	57.84	1.23
Fat percentage	38.17	37.67	37.26	33.56	35.00	1.26

Table 2. Breed differences in meat quality characteritics.

	NoL	DL	GY	P	BL	LSD
Number of animals	80	112	96	92	80	
Rigor value	4.6	5.7	5.1	8.8	8.9	1.0
pH_1 semimem.	6.64	6.51	6.69	6.22	6.17	0.13
pH_1 longiss.	6.55	6.41	6.71	6.21	6.22	0.15
pH ultimate	5.55	5.73	5.73	5.62	5.68	0.10
Transmission value	30.6	31.1	16.4	50.3	49.8	9.6
Fahellpho value	70.7	68.3	61.6	73.5	75.0	4.4
Drip (%)	2.49	1.64	1.39	2.48	1.83	0.33
G6P [1] (µmoles/g)	3.65	3.69	2.23	5.80	5.13	0.62
Lactate[1] (µmoles/g)	10.95	11.31	7.48	18.28	14.04	1.86
Overall score A (4-8)	5.7	5.8	6.7	5.4	5.3	0.4
colour A (4-8)	6.1	6.1	6.9	5.7	5.5	0.4
firmness A (4-8)	5.8	6.1	6.9	5.5	5.7	0.4
leanness A (4-9)	7.9	7.7	7.7	8.4	8.0	0.2
Overall score B (4-8)	5.7	6.2	7.0	5.2	5.4	0.3
Overall score C[2] (1-4)	2.4	1.9	1.5	2.6	2.6	0.3
colour C[3] (1-3)	2.2	1.6	1.4	2.3	2.0	0.3
wetness C[3] (1-3)	1.9	1.5	1.3	2.0	1.9	0.3
texture C[3] (1-3)	1.6	1.2	1.0	1.9	1.7	0.3

[1] n = 55, 69, 53, 61 and 59 respectively
[2] n = 58, 74, 64, 61 and 56 respectively
[3] n = 34, 51, 43, 38 and 32 respectively

Table 3. Mutual correlations between various meat quality and some production characteristics.

n = 460	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Score A	.75	-.76	-.23	.44	.49	.42	-.75	-.70	-.43	-.32	-.61	.22	-.21
2. Score B		-.69	-.32	.49	.55	.33	-.71	-.61	-.50	-.38	-.59	.35	-.38
3. Score C [1]			.21	-.43	-.47	-.38	.77	.64	.35	.24	.60	-.28	.28
4. Rigor				-.62	-.55	.16	.34	.19	.33	.41	.03	-.24	.39
5. pH_1 semimem.					.78	.05	-.50	-.35	-.42	-.42	-.16	.17	-.25
6. pH_1 longiss.						.04	-.54	-.36	-.42	-.40	-.24	.20	-.26
7. pH ultim.							-.28	-.42	-.18	-.05	-.48	.13	-.06
8. Transm. val.								.67	.40	.33	.49	-.28	.33
9. Fahellpho val.									.27	.25	.54	-.22	.27
10. G6P [2]										.67	.23	-.33	.32
11. Lactate [2]											.20	-.39	.38
12. Drip												-.29	.32
13. Growth rate													-.46
14. Meat percentage													

[1] n = 313, [2] n = 297

Table 4. Maximum likelihood solution for 1 and for 2 factors.

	1 factor		2 factors	
	loadings $\hat{\beta}$	variance $\hat{\sigma}^2/\hat{\beta}^2$	loadings $\hat{\beta}$	loadings $\hat{\gamma}$
Rigor	-.31	9.67	+.37	-.57
pH_1 semimem.	+.53	2.60	-.61	+.67
pH_1 longiss.	+.54	2.45	-.62	+.65
Overall score A	+.92	0.18	-.90	-.05
colour A	+.89	0.25	-.88	-.05
firmness A	+.92	0.19	-.89	-.13
Overall score B	+.80	0.55	-.82	+.10
Transmission value	-.83	0.45	+.84	+.03
Drip	-.70	1.04	+.67	+.28
Fahellpho value	-.76	0.74	+.74	+.24
Overall score C	-.89	0.26	+.89	+.25
colour C	-.82	0.49	+.81	+.31
wetness C	-.81	0.54	+.80	+.21
texture C	-.84	0.41	+.84	+.14
G6P	-.43	4.49	+.46	-.36
Lactate	-.41	4.92	+.45	-.38

MEAT QUALITY, HALOTHANE SENSITIVITY AND BLOOD PARAMETERS

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Summary

Phenotypes of the H blood group- and phosphohexose isomerase (PHI) system were determined in 352 Danish, Dutch and Belgian Landrace pigs and related to the pigs' reaction toward a short halothane anaesthesia.

Irrespective of breed all halothane positive (HP) pigs possessed the PHI BB phenotype, while the halothane negative (HN) pigs fell into one or another of the three PHI types AA, AB, and BB. All investigated Dutch and Belgian Landrace pigs possessed the H^a allele, whereas 76 % HP and 30 % HN Danish Landrace pigs possessed this allele.

On the basis of the H system about 76 % of the HP pigs in Danish Landrace can be eliminated, however, 30 % HN pigs are simultaneously eliminated. For the material as whole the corresponding figures were 90 % and 41 %, respectively. The most efficient differentiation between HP and HN pigs was achieved by using both the PHI- and H system, hereby 76-90 % HP pigs could be eliminated, meanwhile the elimination of HN pigs was reduced to 14-20 %.

Introduction

Inferior meat quality (PSE) and PSS are serious problems in pigs, although their importance evidently varies quite markedly from place to place.

A new aspect of this complex problem is the apparent similarity of PSS to the malignant hyperthermia syndrome (MH). Pigs responding to halothane anaesthesia thus appear to be more stress sensitive and liable to exhibit PSE post mortem than non reacting pigs (Sybesma & Eikelenboom, 1969; Harrison, 1972). Accordingly the discovery of accurate methods to identify sensitive pigs would provide a way to select against PSS as well as PSE.

Recent findings of an association between simple criteria like blood groups and enzyme types, and porcine meat quality (Jensen et al., 1976) as well as halothane sensitivity (Rasmusen & Christian, 1976; Jørgensen et al., 1976) do emphasize the importance of immunogenetics and biochemical-genetics within animal breeding programmes. The purpose of the presented work has been to summarize some earlier findings in Danish Landrace (DLR) pigs relevant to this topic as well as present data from a recent investigation on the relationship between blood parameters, like blood groups and enzymes, and halothane sensitivity in Danish, Dutch and Belgian Landrace pigs.

Blood groups and meat quality

Studies of blood group gene frequencies in a breed like DLR consistently exposed to intensive selection is of considerable interest. Provided the traits towards which the selection is aimed are associated with the investigated genetic system (blood groups, enzymes), a further selection is likely to induce shifts in the breed's gene frequencies of those particular systems. In a previous investigation (Agergaard et al., 1976) definite changes were found in the E, G, H and L blood group frequencies. Changes in the H blood group system were the most prominent and consistent. Thus the frequency of the H^a allele showed a steady fall, declining from 0.36 in 1961 to 0.20 in 1976. The regression coefficients on year for the H^a, H^b and H^- alleles were calculated to be -0.0121 ($P<0.001$), -0.0018 ($P<0.05$) and 0.0139 ($P<0.001$), respectively. The significance of these findings is underlined by another Danish investigation demonstrating a close association between the H system and porcine meat colour (Jensen et al., 1976). The main results from this investigation comprising more than 4000 pigs

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during a period of 15 years are given in table 1. On average the frequency of PSE meat (colour score below 2.0) was twice as high in pigs possessing the H^a allele than in those without the H^a allele. A selection against the H^a allele consequently results in a considerable reduction of the PSE frequency within

Table 1. Relationships between the H-types and meat colour score in Danish Landrace pigs during the periods 1961-69 (1) and 1973-75 (2), based on data from Jensen et al., 1976. A colour score below 2.0 is tantamount to the presence of PSE meat.

H-types	No. of animals	Colour score	Animals (%) with a score < 2.0
1.			
a/	833	1.91	37.7
a/-	1039	2.06	24.8
b/	24	2.38	4.2
b/-	200	2.36	6.0
a/b	96	2.26	18.6
-/-	1510	2.33	8.9
2.			
a/	52	1.72	38.5
a/-	121	2.13	28.1
b/	-	-	-
b/-	15	2.43	6.7
a/b	6	2.09	16.7
-/-	176	2.25	14.8
c/	103	2.46	16.5
c/-	118	2.22	21.2
a/c	52	2.01	36.5
b/c	7	2.49	0

the pig population. Such a procedure would also be expected to reduce the incidence of "stress" susceptibility in so far as this somewhat poorly defined phenomenon is related with porcine halothane sensitivity.

Blood groups, enzymes and halothane sensitivity

A relationship has been found between A and H blood group system and halothane sensitivity in pigs (Rasmusen & Christian, 1976). As a linkage earlier has been described between the H blood group system and the enzyme phosphohexose isomerase (PHI) in pigs (Andresen, 1971), investigations of an association between halothane sensitivity and the polymorphic PHI and H blood group systems were carried out.

At an age of 8-12 weeks 268 Danish Landrace, 49 Dutch Landrace and 35 Belgian Landrace pigs were exposed to anaesthesia with halothane and oxygen (Eikelenboom & Minkema, 1974). Using an identical procedure in the present study halothane tests were performed in Denmark (Danish Landrace) as well as in Holland (Dutch- and Belgian Landrace). Sixty two out of 352 pigs were found to be halothane positive (HP).

Blood samples were drawn partly for starch gel electrophoretic determination of PHI phenotypes partly for a blood group test including the H blood phenotypes (Agergaard et al., 1974). All pigs of the present study fell into one or another of the three PHI types AA, AB and BB.

Of particular interest is to note that all HP pigs irrespective of breed possessed the PHI BB phenotype (table 2). This finding shows that halothane sensitivity is closely associated with the PHI phenotype BB.

Table 2. Halothane sensitivity and PHI phenotypes in pigs of Danish, Dutch and Belgian Landrace.

PHI	Danish		Dutch		Belgian		Total	
	HP	HN	HP	HN	HP	HN	HP	HN
AA	0	4	0	0	0	0	0	4
AB	0	99	0	20	0	1	0	120
BB	25	140	9	20	28	6	62	166
Total	25	243	9	40	28	7	62	290
χ^2	17.21		7.60		N.D.		40.92	
P <	0.0005		0.01				0.0005	

However, not all homozygous PHI BB pigs react toward halothane. Thus in Danish Landrace pigs 85 % of the BB phenotypes are HN and for all three breeds jointly this figure amounts to 73 %. A selection against the PHI^{BB} genotype would presumably eliminate all halothane sensitive pigs, but at the same time 57 % of HN pigs would be eliminated too. It is thus necessary to base selection not only on the PHI system, but also on the more definite H locus.

The relationship between the H system and halothane sensitivity is shown in table 3. All Dutch- and Belgian Landrace pigs possessed the Ha factor, while 76 % HP and 30 % HN pigs of DLR possessed this factor. As well in DLR as in the material as

Table 3. Halothane sensitivity and H-phenotypes in pigs of Danish, Dutch and Belgian Landrace.

H	Danish HP	Danish HN	Dutch HP	Dutch HN	Belgian HP	Belgian HN	Total HP	Total HN
a, ac	19	73	9	40	24	7	52	120
b, bc	0	4	0	0	0	0	0	4
c	5	98	0	0	0	0	5	98
-	1	68	0	0	0	0	1	68
Total	25	243	9	40	24	7	58	290
χ^2	21.86		N.D.		N.D.		45.44	
P <	0.0005						0.0005	

whole an excess of HP pigs were found to possess the H^a allele, indicating a close association between halothane sensitivity and the H system in pigs. Breed differences may, however, prevail. Thus in American breeds a high frequency of HP pigs was found within the H type -/- (Rasmusen & Christian, 1976), in contrast to the present study where all -/- animals except one were HN. A selection toward halothane sensitivity can be done on the basis of the H system. In DLR 76 % of HP pigs and in the complete material 90 % of HP pigs could be eliminated by means of the H system. Comparable frequencies of eliminated HN animals were 30 and 41 %, respectively.

The most efficient selection toward halothane sensitivity was achieved by using both the PHI and H phenotypes (table 4). Such a procedure enabled

Table 4. Halothane sensitivity and H-phenotypes in PHI BB pigs of Danish, Dutch and Belgian Landrace.

H	Danish HP	Danish HN	Dutch HP	Dutch HN	Belgian HP	Belgian HN	Total HP	Total HN
a, ac	19	33	9	20	24	6	52	59
b, bc	0	1	0	0	0	0	0	1
c	5	77	0	0	0	0	5	77
-	1	29	0	0	0	0	1	29
Total	25	140	9	20	24	6	58	166
χ^2	27.16		N.D.		N.D.		50.45	
P <	0.0005						0.0005	

us to eliminate 76-90 % of the HP pigs meanwhile the elimination of HN pigs was reduced to 14-20 %.

As a result of these findings it would seem possible by means of these two polymorphic systems to identify halothane sensitive pigs with a considerable accuracy. Furthermore as halothane sensitive pigs have shown a high rate of PSS (Rasmusen & Christian, 1976), the possibility exists that stress susceptible pigs can be pointed out without a previous exposure to halothane.

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INHERITANCE OF M.H.S.-SUSCEPTIBILITY IN PIGS

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Summary

Dutch Landrace piglets aged 6-8 weeks were anaesthetised with halothane for up to 5 minutes in order to be able to classify them as either susceptible (reactors) or non-susceptible (non-reactors) to the Malignant Hyperthermia Syndrome (M.H.S.).

The genetics of this phenomenon was studied by mating pigs of a known phenotype for their reaction to halothane-challenge. Since matings between reactors only resulted in reacting offspring, it was assumed that M.H.S. susceptibility was based on an autosomal recessive gene with complete penetrance. This hypothesis was tested against the segregation ratios among the offspring of matings involving non-reactors and matings between non-reactors and reactors.

In 20 out of 23 matings the ratio between reacting and non-reacting progeny was in agreement with the hypothesis, which led to the conclusion that M.H.S. susceptibility is largely controlled by one major recessive gene. This non-lethal halothane-test therefore offers promising possibilities for selecting against stress-susceptibility and PSE-muscle in pig breeding.

Introduction

Pigs hypersensitive to the anaesthetic halothane show the typical Malignant Hyperthermia Syndrome (M.H.S.) symptoms. A number of studies have shown that a close relationship exists between this hypersensitivity and stress susceptibility and the condition of pale, soft and exudative (P.S.E.) muscle after slaughter (Sybesma & Eikelenboom, 1969; Eikelenboom & Minkema, 1974). The halothane test can be used as an almost non-lethal method to descriminate between stress-susceptible (halothane-positive) and stress-resistant (halothane-negative) animals in 6 to 12 week old piglets.

The literature indicates that the hyperthermia syndrome in pigs following halothane administration is under a rather simple genetic control. However, the suggested genetic mechanisms vary from a single autosomal dominant gene with incomplete penetrance and variable expressivity (Hall et al., 1966), a strongly modified single dominant gene or two dominant genes "acting in concert" (Williams et al., 1975) to an autosomal recessive inheritance with variable penetrance (Christian, 1972). Ollivier et al. (1975) also adopt the hypothesis of a single recessive gene with incomplete penetrance.

A similar syndrome known in man is presumed to be based on a single autosomal dominant gene (Denborough et al., 1962), probably with variable penetrance and expressivity (Britt & Kalow, 1970a, b).

In order to study the genetics of the malignant hyperthermia syndrome more thoroughly a breeding programme was conducted at our institute.

Material and methods

The halothane test as described by Eikelenboom & Minkema (1974) was applied to young Dutch Landrace pigs, belonging to the 6th and last generation of a selection experiment on production traits. Between May 1973 and May 1974 a total of 459 pigs (238 boars and 221 gilts), belonging to four different lines and sired by 33 different boars, were tested for halothane hypersensitivity at about 12 weeks of age. The relationships found in these animals between the reaction upon the test and production characteristics are reported by Van Eldik (1975) and reviewed by Eikelenboom et al. (1976).

Planned matings between a number of these animals of known phenotype for the halothane reaction which were kept for breeding, were performed between April 1974 and July 1975. The offspring of a total of 52 successful matings were tested for halothane hypersensitivity at 6 to 8 weeks of age.

With the results of the matings the hypothesis was tested that halothane sensitivity is based on a single autosomal recessive gene. Under this hypothesis 3 genotypes can be distinguished: NN and Nn, both reacting negative and nn reacting positive. Negative animals can be either homozygous or heterozygous. Matings involving heterozygous parents, recognised as such on the basis of the results of previous matings, are regarded as unconditional or a posteriori-matings. In this case the expected number of positive offspring is $\frac{m}{4}$ for Nn x Nn-matings and $\frac{m}{2}$ for Nn x nn-matings, where m = total number of offspring per litter.

In the early phase of the planned matings in particular some of the heterozygous negative parents were spotted as such because they produced one or more positive progeny in their actual litter. The expected number of positive progeny from these conditional or a priori-matings is:

$\frac{\frac{m}{4}}{1 - (\frac{3}{4})^m}$ for Nn x Nn-matings, and

$\frac{\frac{m}{2}}{1 - (\frac{1}{2})^m}$ for Nn x nn-matings.

If the hypothesis is true the number k of positive progeny follows a binomial distribution, or a conditional distribution derived from the binomial distribution, given $k > 0$. Hence, critical levels for testing the hypothesis for each mating separately can be obtained from cumulative binomial probability tables.

The results of both a priori and a posteriori matings of the same type were pooled by estimating the mean difference $\bar{d}$ between observed and expected number of positive progeny, weighing each individual difference d_i by the reciprocal of its variance. If the hypothesis is true the standardized weighted mean difference $\frac{\bar{d}}{\sigma_{\bar{d}}}$ is approximately normally distributed with mean 0 and standard deviation 1.

The variance of the deviations $(d_i - \bar{d})$ at unit weight is approximately distributed as $\frac{\chi^2_{I-1}}{I-1}$, where I is the number of matings pooled, providing a test for heterogeneity.

Results and discussion

Since all 9 matings between positive parents resulted in litters with positive piglets only (Table 1A), it was assumed that halothane sensitivity might be based on a single recessive gene with complete penetrance. This hypothesis was tested against the results of the other matings. In only one of the 9 litters from a priori matings between heterozygous negative parents, did the segregation ratio observed not fit with the hypothetical one (Table 1B1). These negative parents were regarded as heterozygotes, since they produced one or more positive progeny in their actual litter.

There were another four matings between negative animals, already known to be heterozygotes. They were recognized as such because they had either produced positive offspring in a previous litter or they themselves were produced from a mating between a negative and a positive parent. In all 4 a posteriori-matings the number of positive offspring was in agreement with the expectations (Table 1B2).

The combined test of all 13 Nn x Nn-matings also agreed with the single recessive gene hypothesis. Furthermore no significant heterogeneity could be detected (Table 2.1).

From the 3 a priori matings between heterozygous negative and positive parents 2 confirmed the hypothesis. In one case a significant shortage of positive offspring was found (Table 1C1). In 6 out of 7 a posteriori Nn x nn matings the observed number of positive offspring was in accordance with the expected number, but in one case a very significant shortage of positive progeny occurred (Table 1C2). Also the pooled test of the 10 Nn x nn matings yielded a significant shortage of positive offspring as well as a significant inconsistency between the results of the individual matings (Table 2.2).

Another 16 matings between negative parents of unknown genotype were performed, all resulting in negative offspring only. These matings are of no value for testing the hypothesis. One of the negative boars involved was mated with 5 negative sows and produced negative offspring only. Later on 3 out of these 5 sows in fact turned out to be heterozygous, so it was highly likely that the boar was homozygous negative NN. Mating this boar with 4 positive sows confirmed this expectation, since all 4 litters consisted of negative piglets only.

When interpreting the results of the test matings it should be born in mind that the reaction to the halothane test is not always 100% clear cut. Two kinds of mistakes are possible. A reacting animal may be wrongly scored as negative, because of a delayed reaction. Such a reaction was sometimes observed in runt pigs, sick animals and after a period of starvation. A negative animal may be wrongly scored as positive, since sometimes piglets show muscular stiffening at the beginning of the test which, however, disappears when halothane administration is continued.

On the whole the results of the matings agree rather well with the hypothesis that the Malignant Hyperthermia Syndrome is based on a single recessive gene with complete penetrance. It seems therefore justified to conclude that a major recessive gene is involved. This makes it possible to considerably decrease the incidence of stress susceptibility in a pig population by a straight forward selection against halothane-positive animals. However, at low frequencies this phenomenon will act as a recessive genetic defect. In order to eliminate this gene completely it will be necessary to progeny test the breeding animals to detect possible carriers of the gene. This is a time consuming and rather expensive procedure, which usually does not pay since a recessive gene with a very low frequency will not do much harm to the population.

It could be possible that the gene controlling halothane sensitivity is behaving differently in different populations. Our results indicate a complete penetrance, but Ollivier et al. (1975) conclude an incomplete penetrance in studying this syndrome in a French Piétrain-line. Also the incidence of halothane sensitivity may vary widely in different populations, as shown in Table 3, which gives the preliminary results of testing 6 lines of 6 different breeds at our experimental farm

(Cöp & Buiting, 1976).

Eikelenboom & Minkema (1974) as well as Ollivier et al. (1975) have found that the positively reacting animals have a higher meat content. The same conclusions can be drawn from the breed differences in M.H.S.-frequency in Table 3. Both Piétrain and Belgian Landrace are very M.H.S.-susceptible and have a superior muscularity as well as an inferior meat quality. Although it has not yet been proved it seems that the gene for halothane sensitivity has a pleiotropic effect on muscularity and meat quality. Because of their superior meat content breeds like Belgian Landrace and Piétrain could be especially useful in pig breeding schemes. They could provide an element in a crossbreeding programme, especially as a terminal sire line. When they are crossed with a homozygous negative dam line all offspring would also be negative if the recessive gene hypothesis holds. The progeny would not suffer from a higher stress susceptibility, but would still benefit from the better muscularity of the sire line, because muscularity seems to be inherited in an additive manner which can be seen from most crossbreeding experiments in pigs.

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Table 1. Results of matings with regard to halothane sensitivity

Mating type	Mating number	Number of offspring	Number of positive offspring observed	expected
A. Positive x Positive	1	8	8	
	2	4	4	
	3	10	10	
	4	11	11	
	5	6	6	
	6	9	9	
	7	5	5	
	8	9	9	
	9	10	10	
B1. Heterozygous negative(Nn) x Heterozygous negative(Nn) a priori	10	12	3	3.10
	11	3	1	1.30
	12	13	4	3.33
	13	7	5*	2.02
	14	8	1	2.22
	15	3	1	1.30
	16	13	4	3.33
	17	6	3	1.82
	18	10	5	2.64
B2. Heterozygous negative(Nn) x Heterozygous negative(Nn) a posteriori	19	8	2	2
	20	7	2	1.75
	21	9	2	2.25
	22	10	1	2.5
C1. Heterozygous negative(Nn) x positive(nn) a priori	23	10	3	5.01
	24	7	2	3.53
	25	9	1*	4.51
C2. Heterozygous negative(Nn) x positive(nn) a posteriori	26	10	0**	5
	27	9	5	4.5
	28	6	3	3
	29	8	2	4
	30	3	2	1.5
	31	13	6	6.5
	32	8	5	4

* significant at 5% level
** significant at 1% level

Table 2. Pooled results of matings.

Mating type		
2.1. Nn x Nn (13 matings)	Pooled test $\frac{\bar{d}}{\sigma_{\bar{d}}} = +.6513$	$P(\lvert\underline{\chi}\rvert > 0.6513) = .26$ n.s.
	Heterogeneity test (I - 1) Var $(d_i - \bar{d}) = 17.6245$	$.1 < P(\underline{\chi}^2_{12} > 17.6245) < .2$ n.s.
2.2. Nn x nn (10 matings)	Pooled test $\frac{\bar{d}}{\sigma_{\bar{d}}} = -2.2385$	$P(\lvert\underline{\chi}\rvert > 2.2385) = .01$ significant
	Heterogeneity test (I - 1) Var $(d_i - \bar{d}) = 18.2807$	$.01 < P(\underline{\chi}^2_{9} > 18.2807) < .05$ significant

Table 3. Frequency of halothane sensitivity in 6 different lines from experimental station "Bantham" (Cöp & Buiting, 1976).

Line	Number of animals tested	Percentage of reactors
Belgian Landrace	50	84.8
Dutch Yorkshire	90	0
Piétrain	53	100.0
Duroc	107	0
Hampshire	116	1.7
Dutch Landrace	133	12.8

FREQUENCY OF THE MALIGNANT HYPERTHERMIA SYNDROME (MHS) IN SOME FRENCH PIG POPULATIONS : PRELIMINARY RESULTS

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Summary

In France five pig populations are currently being investigated for MHS by means of a 5-minute halothane anesthesia, applied over a live weight range of 20 to 35 kg. These populations are :(1) a "normal" Piétrain line (PA), (2) a Piétrain line selected for muscular hypertrophy (PB), (3) the Belgian Landrace breed (LB), (4) the French Landrace breed (LF) and (5) the Large White breed(LW). Whereas in the first two populations (PA and PB) all piglets weaned are halothane-tested, in the last three ones the test is only applied to boars entering performance-testing stations. Muscular development is also assessed visually at the time of the test.188 PA, 127 PB, 85 LB, 98 LF and 102 LW have so far been tested (over the period march-june 1976). The frequency of MHS is 34±3, 47±4, 55±5, 18±4 and 0 percent respectively in the five samples studied. Mortality due to the test has been 8.5 per cent among MHS pigs, with similar percentages in the four populations affected. Assuming that MHS is determined by one autosomal, recessive gene, the gene frequency and penetrance can be estimated from the distribution of affected animals over the litters tested. The estimations in the 4 affected populations are respectively 0.76± 0.12, 0.83±0.14, 0.83±0.16, 0.38±0.10 for gene frequency and 0.59±0.17, 0.69±0.23, 0.80± 0.31, 1.30±0.65 for penetrance. A slight upward bias is likely in the LB gene frequency estimation, as a result of a possible selection by the breeder on conformation within litter. In the LW breed, only an upper limit can be estimated for gene frequency, which is 2.2 per cent at the 5 % probability level, assuming full penetrance. These results confirm the strong genetic association between MHS and muscular development as it can be judged visually and selected for by the breeder at weaning time, and suggest that such a selection might be at the origin of the Piétrain and similar breeds.

Introduction

Halothane-induced malignant hyperthermia syndrome (MHS) in pig is presently studied in many countries, often in relation with porcine stress syndrome (PSS) and pale,soft and exudative meat (PSE). In France our first investigations on MHS were made in an experimental Piétrain herd (Ollivier et al.,1975; Monin et al., 1976). However, MHS was also reported in a number of pig breeds over the world and we have conducted an experiment to obtain informations on (1) the frequency of MHS in the breeds used in France, (2) the relationship between MHS and various production as well as biochemical characteristics.Preliminary results of this study are given here.

Material and methods

Over the period march-june 1976, a total of 600 pigs from five populations have been tested for MHS by a 5-minute anesthesia with halothane, applied over a liveweight range of 20 to 35 kg. Table 1 gives the numbers of pigs, sires and dams in each of the five samples.

Table 1.Numbers of pigs,sires and dams in each sample.

Sample	No. of pigs tested	No. of sires	No. of dams
PA	188	7	26
PB	127	7	21
LB	85	35	54
LF	98	31	59
LW	102	46	65

Data about the Piétrain breed were obtained in two separate lines selected since 1973 in the I.N.R.A. experimental herd in Avord,these animals being the result of 2 generations of selection. In the A-line (PA) the objective of selection is to improve postweaning growth rate and to reduce backfat thickness. The B-line (PB) is selected for an increased muscular hypertrophy and the criterion of selection is a visual score given at around 20 kg according to the method described by Ollivier & Lauvergne (1967). In these two populations all piglets surviving until 20 kg (males and females) were halothane-tested and scored for conformation at the time of the test. In the 3 other populations, Belgian Landrace (LB), French Landrace (LF) and Large White (LW),we have tested samples of young boars entering performance-testing stations. Four "batches" of boars in two stations (Gannat and Le Transloy) are included, two or three breeds

being represented in each "batch". Muscular development was also assessed visually using the same method as for Piétrain pigs.

In each sample, assuming that MHS is due to an autosomal recessive gene, we have applied a method which gives a simultaneous estimation of its frequency (q) and of its penetrance (w), knowing the number of affected animals observed in a series of sire-progenies, each sire-progeny being itself subdivided into a variable number of full-sib families (Lefort et al., 1975).

Least-square means of the conformation score were calculated for each population and, in the four affected populations, for the susceptible and non-susceptible pigs.

Results and discussion

Table 2 shows the percentage of pigs which exhibited MHS and the mortality rate due to the test among MHS pigs in the 5 samples.

Table 2. Frequency (F) of MHS and mortality (M) due to the test among MHS pigs in the 5 populations.

Population	F±S.E.(p.cent)	M (p.cent)
PA	34±3	7
PB	47±4	6
LB	55±5	13
LF	18±3	11
LW	0	0

The frequency of MHS is highest in Belgian Landrace breed, followed by the Piétrain line selected for muscular hypertrophy and by the "normal" Piétrain line. The frequency of MHS is much lower in French Landrace than in Belgian Landrace. The frequency in LF is about the same as that found by Eikelenboom & Minkema, 1974) in Dutch Landrace. None of the Large White pigs exhibited MHS.The average mortality due to the test has been 8.5 p.cent among MHS pigs, with rather similar percentages in the four populations affected.

Estimated frequencies and penetrances of the postulated recessive gene responsible for MHS are reported in Table 3 for PA, PB, LB and LF populations.

Table 3. Frequency (q) and penetrance(w) of the postulated recessive "MHS" gene in the 4 affected populations.

Population	q ± S.E.	w ± S.E.
PA	0.76 ± 0.12	0.59 ± 0.17
PB	0.83 ± 0.14	0.69 ± 0.23
LB	0.83 ± 0.14	0.80 ± 0.31
LF	0.38 ± 0.10	1.30 ± 0.65

In the Large White breed, only an upper limit can be given for gene frequency,which is 2.2 per cent at the 5 p.cent probability level, assuming full penetrance.

The gene frequencies in the PA, PB and LB samples do not differ significantly, but they significantly exceed the LF gene frequency, which itself exceeds the LW.

Estimates of penetrance have large standard errors, especially in the two Landrace samples. The estimates relative to the Piétrain lines are in good agreement with our previous estimate ($\hat{w} = 0.69 \pm 0.25$)from another sample of Pietrain pigs (Ollivier et al., 1975). Altogether these data indicate that the penetrance of the gene responsible for MHS is likely to be high, but possibly lower in Pietrain than in French Landrace. The trend observed over the 4 penetrances of table 3 might indicate real breed differences. These could be a consequence of selection for low penetrance applied in Piétrain for several generations. Such a selection could not have been as effective in breeds like LB and LF, where the gene has been introduced more recently.

The five populations under study also greatly differ with respect to muscular development as assessed by a visual score(Table 4).

Table 4. Population means for a visual score of muscular development (1)

Population	mean	±	S.E.
PA	3.99	±	0.13
PB	5.87	±	0.19
LB	7.02	±	0.22
LF	2.06	±	0.17
LW	1.33	±	0.16

(1) the score is proportional to the degree of muscular hypertrophy and goes from 0(null) to 12 (extreme).

The 5 samples rank in the same order for frequency of MHS (table 2) and for muscular development (table 4), which indicates a strong association between MHS and conformation. This relationship is further supported by the significant divergence between the 2 Piétrain lines - as well in incidence of MHS as in muscular development - a a consequence of 2 generations of selection for different goals in the 2 lines.

The same relationship is apparent within line or breed, in the PA, PB and LF samples, as MHS - susceptible animals have a better muscular development than normal animals (table 5). However,in the LB sample, no significant effect of type of reaction to halothane is found for the muscular hypertrophy score. This apparent exception might be due to a selection on conformation by the breeder when he chooses young boars to be

sent to the performance-testing stations. As a consequence, the LB sample would not be a random sample of the breed with regard to MHS sensitivity as well as to muscular development. The apparently equal muscular development in the 2 types of LB pigs also indicates that extreme muscularity may exist in non-MHS pigs, and suggests that the MHS gene may be, at least partially, dominant for muscle development. This would imply that a great number of non-MHS pigs in the LB sample are in fact heterozygous for the MHS gene. Another consequence of this non-randomness of the LB sample is a slight upward bias in the gene frequency estimation given for that breed in table 3. Such a choice by the breeder is likely to be less effective in the LF and LW breeds where muscular development is much less conspicuous at weaning time

Table 5. Means (± S.E.) for score of muscular development by type of reaction to halothane anesthesia within the four MHS - affected populations.

Population	type of reaction (+)	type of reaction (-)	Significance of the difference
PA	4.63±0.23	3.64±0.15	$P < 0.01$
PB	6.43±0.26	5.34±0.27	$P < 0.01$
LB	7.05±0.34	7.00±0.36	$P > 0.05$
LF	3.26±0.41	1.80±0.22	$P < 0.01$

These preliminary results indicate important breed differences in incidence of MHS among the 4 french breeds studied. These differences correspond to different frequencies, and also possibly to different penetrances, of the postulated recessive gene responsible for the abnormality. These results also confirm the strong genetic association between MHS and muscular development, as it can be judged visually and selected for by the breeder at weaning time. Such a selection might indeed be at the origin of the Piétrain and similar breeds. However, differences exist between breeds in apparent muscular development at weaning time which can not be attributed to the MHS gene.

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SOME PRELIMINARY OBSERVATIONS ON THE INHERITANCE AND APPLICATION OF HALOTHANE-INDUCED MHS IN PIGS

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Summary

The incidences of MHS in 266 Pietrain/Hampshire crossbreds and 157 pure Norwegian Landrace given a 3-minute halothane test at 5-15 weeks of age were 20% and 5% respectively, while no positive reactions were detected in 85 Duroc and 66 Hampshire purebreds. When the Pietrain/Hampshires were subsequently divided into two lines of roughly 25 gilts each, one containing positive or doubtful reactors (MHS-susceptible line) and the other negative reactors (MHS-resistant line), the incidences of positive halothane reaction in the resulting progeny were 78% and 12% respectively. The incidence of positive reaction in the progeny of 14 inter se matings of definite positive reactors in the MHS-susceptible line was 96%. From 335 Pietrain/Hampshires given a second halothane test 3-4 weeks after the first, the probability of misclassifying a pig on one test was estimated at 6%.

Introduction

The range of stress-related defects generally known as Porcine Stress Syndrome (PSS), which includes Pale, Soft and Exudative (PSE) meat and "sudden death", is a source of economic loss in pig production. Now that a relationship has been demonstrated between PSE and halothane-induced Malignant Hyperthermia Syndrome (MHS) (Eikelenboom & Minkema, 1974), interest has grown in using halothane reaction as a method of screening young PSS pigs from breeding programmes before starting on expensive performance test. PSS is clearly to some extent hereditary, and there are indications that both MHS (Ollivier et al., 1975) and PSE (Jensen, 1974) might have a simple recessive form of inheritance. This report gives some preliminary results from an evaluation of the halothane test for genetic improvement programmes.

Methods

Young pigs were given a 3-minute halothane test at between 5 and 15 weeks of age. The halothane was administered in pure oxygen flowing at 2-3 litres per minute from a semi-open circuit apparatus via a tight-fitting face mask. The initial concentration of halothane (4-8%) was chosen so that the eye-reflex was lost within one minute, and thereafter reduced to the point where anaesthesia was just maintained. All pigs were transported from their pens to the operator for testing. Reactions were scored as either "positive", "doubtful" or "negative"; a positive MHS reaction defined as obvious rigidity of the hind leg. The face mask was removed as soon as a positive reaction was detected.

The pigs tested were sampled from a composite line (PH) containing 50% Pietrain and 50% Hampshire, and from purebred populations of Norwegian Landrace (NL), Duroc (D) and Hampshire (H). In order to show the effectiveness of selection in changing the incidence of MHS, and to throw more light on its mode of inheritance, the halothane-tested PH population was divided into two lines: one containing positive or doubtful reactors (MHS-susceptible line) and the other negative reactors (MHS-resistant line). Progeny from the two lines were halothane-tested early in 1976.

As a check on the reliability of the halothane test, samples of PH pigs were given a second identical test 3-4 weeks after the first. The probability (p) of misclassifying a pig on one test was then calculated from the proportion of disagreements (d) between the outcome of first and second tests, where $d = 2p(1-p)$ (D.I. Sales, personal communication), counting doubtful reactions as negative and assuming an equal frequency of errors among positive and negative reactors.

Results and discussion

The incidences of positive MHS reaction (Table 1) were 19.9% in the unselected PH and 4.5% in the NL, while no positive reactions were detected in the D and H samples. These results were much as expected from the known relative stress-susceptibility of the breeds, and compare with reported incidences of 13% in Dutch Landrace (Eikelenboom & Minkema, 1974) and 28% in the Pietrain in France (Ollivier et al., 1975), suggesting that the PH is more stress-susceptible than the Dutch Landrace and less so than the pure Pietrain. The zero incidence in the H sample will require verification, but would indicate that all the stress-susceptibility in the PH originates from the Pietrain.

Table 1. Incidence of positive MHS reaction by breeds.

Observation	Breed (see text)			
	PH	NL	D	H
No. measured	266	157	85	66
No. positive reactions	53	7	-	-
% Incidence	19.9	4.5	0	0
No. litters measured	35	24	13	8
No. litters affected	27	4	-	-
No. sire families measured	15	8	5	5
No. sire families affected	13	2	-	-
No. deaths within 24 hrs.	5	1	-	-
Mortality (% of positive reactors)	9.4	14.3	-	-

77% of measured PH litters contained affected individuals, and an average 26% of pigs within the affected litters were MHS positive (Table 1), a pattern which might be explained by a monogenic recessive form of inheritance. Mortality in these early experiments was fairly high with 9% of PH positive reactors dying within 24 hours of testing. This figure later declined to less than 8% in the selected lines.

Table 2. Incidence of positive MHS reaction in initial PH population (FP), MHS-susceptible (MS) and MHS-resistant (MR) lines.

Line	No. litters	No. pigs	Incidence (%)	Gene freq.*
FP	35	266	20	0.45
MS	24	160	78	0.88
MR	27	180	12	0.34

* approximation (see text)

The results of selection for and against MHS in the PH population are shown in Table 2. In one generation the incidence of positive MHS reaction increased from 20 to 78% in the MHS-susceptible line, and decreased from 20 to 12% in the MHS-resistant line. A single generation of two-way selection therefore produced a divergence in incidence of 66%. Simple estimates of gene frequency on a single recessive gene model, calculated as the square root of the incidence, are given for illustration, but take no account of family structure or possible reduced penetrance (Ollivier et al., 1975).

Table 3. Incidence of positive MHS reaction from inter se matings of definite positive (R) and definite negative (NR) reactors.

Mating	No. litters	No. pigs	Incidence (%)	Gene frequency	
				obs.	exp.
RxR	14	75	96	0.98	1.00
NRxNR	19	125	12	0.35	0.31

The incidences of MHS from inter se matings of definitely identified positive reactors and definite negative reactors from the two PH lines are given in Table 3. 96% of the progeny from positive by positive matings were themselves MHS positive, so that the empirical observed gene frequency approached its expected value of 1.00 on a monogenic recessive model. 12% of the progeny from negative by negative matings were MHS positive, and once again the observed and expected gene frequencies were in fairly close agreement. These results tend to support the hypothesis that halothane-induced MHS is caused by a single recessive gene, but a more thorough genetic analysis has been started by Dr. P. Bampton.

Table 4. Results of repeated halothane tests (PH lines).

Observation	Number	%
Pigs measured twice	335	-
Positive at both tests	83	25
Positive at 1st test only	24	7
Positive at 2nd test only	11	3
"Disagreements"	35	10

In the PH pigs given a repeat halothane test 3-4 weeks after the first, the frequency of disagreements between the outcomes of first and second tests was 10% (Table 4), from which the probability of wrongly predicting an animal's true genotype for MHS-susceptibility on one halothane test was estimated at 6%. Correct selection decisions on MHS-susceptibility would therefore be expected on 94 out of every 100 pigs tested. It can be shown that, when the objective is to select only stress-resistant individuals from a population in which the incidence of MHS is 20%, the probability of correctly identifying a negative reactor on one test is 98%.

The preliminary experiments reported here indicate that a rapid halothane test is both easy to conduct and reliable. The results support the hypothesis of simple recessive inheritance and show conclusively that selection can be effective in changing the incidence of MHS. However, before the success of large scale halothane testing as a countermeasure for PSS can be predicted, the exact genetic association between PSE and MHS must be known. Further, the relative merits of other prediction methods such as blood-typing (Rasmusen & Christian, 1975; Jensen et al., 1976) or serum enzyme analysis, or even direct selection on PSE (Jensen, 1974), are unclear. In breeds where the incidence of PSE is low, as in Britain, the economic loss resulting from PSS is difficult to define, and the degree to which genetic control measures are applied may depend on whether or not the incidence of PSS is likely to increase as a consequence of continued selection for lean meat production. It will therefore be important to obtain accurate estimates of the genetic correlation between the various components of PSS and all the other economically significant traits which are taken into account during selection.

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VIEWPOINTS ON THE PORCINE LEG WEAKNESS SYNDROME

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The viewpoints given in this lecture on leg weakness in pigs are based on scientific experiments and practical experience, obtained by own investigations and experience, literature studies and personal communication with people interested in pig production. All the conclusion will not be documented by results or references, as done in strictly scientific articles. However, list of references are available by the author.

Leg weakness

Definition

Leg weakness is a syndrome. A syndrome is a complex of symptoms. A symptom might have one special etiological factor, but same symptom might also have different etiological factors or is formed by interaction of several etiological factors. Thus, leg weakness is far from an exact diagnosis. It is an evaluation of the pigs ability to move, usually based on visuell impression. In own investigations, a scale from 3 to 8 was used. 3 was pigs not able to arise, 4 severe degree and 5 mild degree of leg weakness, 6 satisfactory movements, 7 good and 8 very good movements. When judging, emphasis was put on the pigs ability to get to its feet, whether it could trot, how easily and springy it moved, whether it had a stiff or swaying gait, and whether it slipped about. Thus, pigs without training in walking, or with genetically "poor" movements might be put into the legweak group, whithout having any disease or lesion. In our experiments, arthritis and serious hoof lesions were uncommon. Elsewhere theese will complicate a constitutional ranking. It is also essential to be aware that pig breeds might have their special gait, and take that moment into consideration.

Incidence

In a material consisting of 373 Norwegian landrace pigs 1,3% were not able to arise at 100 kg live weight, 16,9% had severe degree leg weakness, 30% mild degree, 30,6% satisfactory movements, 20,1% good movements and 1,1% very good movements. At a Norwegian testing station for boars 16% had severe degree leg weakness in 1969/70, in 1973/74 the incidence was 5%. Two main reasons for this improvement seem to be logical. The selection against functional bad exterior conformation was intensivated, and, in spite that growth rate has increased, feed rate has not been intensivated (better feed conversion). It seems therefore that the boars today in fact are fed more restricted than earlier.

The practical significance of leg weakness is greatest in young breeding animals. In 1969/70 35% of the boars under 1½ years old were culled because of leg weakness, while the culling caused by leg weakness in older boars was 12%. Now the figures are lower in the youngest group, mainly I suppose because young boars today not are tried in breeding if they have severe to mild degree leg weakness. This apparent improvement will in my opinion not continue.

In a Norwegian experiment landrace are selected in two directions for growth rate and backfat thickness. In the rapid growing line, leg weakness was responsible for the culling of 20,5% of the sows. In the slowgrowing line the figure was 13,8%. At that time the average backfat thickness at 90 kg live weight was 22,5 mm in the rapid growing line and 37,4 mm in the slowgrowing line. The difference in culling rate caused by leg weakness is marked, but the results show that older type of pigs also have leg problems.

Etiology

When seeking the main reasons for bad movements, one might approach the problem in different ways. The most used method is a postmortem investigation of the diseased pigs. Then one will focus the gross lesions and factors that are easily available, and may be those traditionally connected to movements. This leads to the bones and joints. We know however, that some young pigs have a poor gait without having lesions of supposed seriousness in joints. Therefore we sometimes need to repeat which organs are taking main part in locomotion:
nervous system, muscles, tendons, ligaments and their incertions, joints, bones and hooves. Overloading of liga-

ments, which we know will give pain, seem not to be thoroughly investigated, nor the maturing and firmness of collagen in rapid growing pigs. Joint cartilage and bones are thoroughly morphologically investigated, the importance of the hoof status seem to be more and more accepted, and "weak" incoordinated muscles have also been given responsibility for poor movements. It is specially in the pigs around slaughter age the etiological specter for leg weakness is so wide. In breading pigs, were the problem in my opinion is greatest, both economically and as an animal protection problem, the diagnoses osteochondrosis, arthrosis, epiphyseolysis, intervertebral disc degeneration and spondylosis are in Norway responsible for about 75% of serious affected legweak pigs. In boars the elbow and stifle joints count for about 30%, the lumbar intervertebral joints for about 30% and the hip joint for about 15%. As osteochondrosis often progress into (osteo) arthrosis, and osteochondrosis also might give rise to epiphyseolysis, it must be concluded that osteochondrosis is essential when discussing leg weakness in pig. Nevertheless we should have in mind that arthritis, periarthritis, intervertebral disc inflammation, hoof lesions, myopathies, fractures and ligamental ruptures etc. also occur. The share of theese diagnoses will vary from herd to herd and country to country.

Relation to osteochondrosis

Osteochondrosis, a disturbance of the endochondral ossification in as well joint cartilage as in epiphyseal plates, is a generalized disease. Osteochondrosis can heal completely, be repaired or progress into (osteo) arthrosis. The incidence in modern pigs is nearly 100%, while an incidence of about 95% was found in slowgrowing backfat pigs at 90 kg live weight. This shows that the skeleton even of slowgrowing pigs is very prone to injuries, as other investigations also have shown. Most of the severe cases occur at certain spots in the medial condyle of femur and humerus and in the intervertebral joints. "General weakness" obviously is present, but local conditions in the joints must be dicisive for the further development of the lesions. The lumbar part of the spinal column seem to have become a weak skeletal part. The incidence of lesions in this part in modern Norwegian landrace were 14,3% at 90 kg and about 45% at 100 kg, in short, slowgrowing landrace, 2,7% at 90 kg, and in Yorkshire 4,4% at 100 kg. In modern breeding landrace pigs the incidence was about 75%. It is essential to make clear that leg weakness and osteochondrosis not necessarily are the same. Clinical and postmortem investigations of the same pigs show that only osteochondrosis of severe degree ("open" lesions) surely give clinical symptoms.

Main factors connected to leg weakness

I. Feeding factors

a) Energy intake

Most research results show that high feed level gives rise to clinical locomotory problems. Concerning osteochondrosis, however, the results are not so clear. In own investigations there was only slight tendencies towards differences in degree of osteochondrosis between the feed level groups. The average age at slaughter at 100 kg live weight was 176 and 202 days respectively. In experiments where the pigs are slaughtered at a certain age instead of a certain weight, there usually are differences in severity of osteochondrosis between groups.

b) Mineral, vitamin and protein supply

If the minerals Ca and P are given in a resorpable form, and the vit. D supply is as recommended today, moderately high or low level of Ca and P seem not to have any noticeably influence on incidense and degree of leg weakness and osteochondrosis. However, 1,0 to 1,2% Ca and about 1,0% P in the feed give histomorphologically seemingly more optimum structure of the spongious bone tissue and higher ash percentage in the bones than about 0,7% Ca and 0,6% P or unbalanced mineral levels in the ration. The minerals Mg, Cu, Zn and Mn and vit. A and D within wide limits seem not to have any influence on incidence and degree of osteochondrosis. Some reports give Se and vit. E some effect in preventing leg weakness, by preventing myopathies. A point in the mineral disussion is whether breeding pigs, to be sure they will get well mineralized skeleton, should be given high level Ca and P when growing up. Further, as the pigs get a more and more efficient feed conversion one might have to reestimate the concentration of vitamins and minerals in the ration. Quantity and sources of protein inside wide limits seem not to have any influence of pract-

ical significanse on leg weakness or osteochondrosis.

II Environmental factors

a) Exercise

It is a generally held opinion that exercise has a favourable influence on locomotory ability in pigs. What mechanism that are at work, are not easy to say. In own experiments the degree of osteochondrosis was not lowered by exercise, so the effect must concern the other organs taking part in locomotion. It is reasonable to suppose that muscle strength increases with exercise. It also seemed that the ability to use the proper muscles at the right time was concid-erably more developed in the exercised animals. This should indicate a training of the nervous system as well.

b) The floor, bedding and equipment

More and more attention has been paid to the lesions in the hooves of the pigs. Further, it is shown that hoof horn growth usually exceeds the wear. Hoof care in breeding pigs is undoubtally an important and neglected field. New floors with small sharp prominences will give hoof lesions and possibilities for infections. Too smooth floor are often slippery and give rise to accidents like ligamental and muscle ruptures. Too wide openings in slatted floors might also lead to accidents, the same with openlying mechanical manure handling systems. Outdore life or an effective bedding, were it is dry enough to prevent infections, moist enough to prevent hoof fissures, soft enough to be lenient to the hooves but giving the needed friction to walk safely, combined with good hoof care, might be said to be desirable.

c) Transportation and management

Under and after transportation many breeding pigs have got severe locomotory problems. Sometimes there are obvious accidents as fractures or ligamental ruptures, sometimes a more obscure etiology. In slaughter pigs one often finds bleedings in ligaments. In own experiments the serum transaminases GPT and GOT was highered and seemed to vary with the environmental factors in connection with transport and slaughter. Whether the muscles was affected to a degree that would give clinical symptoms is a question that are not answered. We do not exactly know the value of it, but Norwegian breeding pigs are often given a high dosis of vit. E or vit. E and Se before long distance transportation. Pen size and number of pigs pr. pen are of interest when discussing management. One young pig each pen, specially if combined with high level feeding, most often give locomotory problems. This is thought to be because of lack of motion. Too crowded in the pen might also give locomotory problems. Besides, when boars are starting to mount each other in a pen, they usually will have a period with stiff gait. Moderate use and a suitable pen for young breeding boars are of great value. Gilts will have a "weak" locomotory apparatus at the first farrowing. Moderate exercise during the gestation will improve the condition of both gilts and sows.

III Hereditary factors

According to the scarse literature on the subject, the heritability of leg weakness score is considered to be low, about 0,2. The highest documented figure is 0.5, which was concidered to be calculated too high. Race, line, sire group and litter differences in leg weakness, osteochondrosis and different parameters connected to these indicate however, that heredity plays a real role in the leg weakness syndrome. One of the basic questions is which criteria to use when trying to select for good movements, or against leg weakness.

a) Growth rate

Growth rate is a very important factor in the leg weakness syndrome. In own investigations growth rate to 60 kg live weight was highly correlated to locomotory problems at 100 kg. Dayly weight gain for pigs getting 3 in gait score (not able to arise) was 775 from about 20 to 60 kg, gait score 4:722 g, gait score 5:662 g, gait score 6:659 g, gait score 7:644 g and gait score 8 (very good movements): 556 g. From 60 100 kg the relation was not so marked. In this results both genetically and feeding determined rapid growth are put together.

There is also relation between growth rate and osteochondrosis. The incidence seem not to be so much lowered in slowgrowing pigs (from 99% to 95% in own investigations on genetically different pigs) but the degree or severity of the lesions is higher in rapid growing pigs. This difference in degree might be used as an explanation of why there is so marked relat-

ion between growth rate and leg weakness. Nevertheless, in my opinion there must be other factors acting too, and it seems reasonable to draw attention to the ligamental apparatus, collagene maturing and the muscles, as also has been done.

As growth rate is one of the most important economical factors in pig production, I think a general reduction of this, or a stop in the evolution, should not be done before other possible preventive initiatives have been tried. Growth rate in pigs that are bound to be breeders is a question about selection systems.

b) Exterior conformation

There is considerably variation in the exterior of pigs, both between and within breeds. A long back and a special shape of the hams have been paid attention in connection with leg weakness. Norwegian landrace is long. In some lines there also is very broad hams, often combined with narrow lumbar region, bow hind legs and a swaying gait. These pigs have a heavy action and seem to be "weak". The mechanism for this is not fully understood. However, as the lumbar part of the spinal column is the skeletal part that has become "weakest" in modern Norwegian landrace, it is reasonable to pay special attention to the lenght and the overline of the pig, and also try to get rid of the extreme shaped lumbar region and hams. In some breeds we do not find this exterior conformation, so there at the moment, it is not of interest.

Concerning the distal part of the legs, there is not any new viewpoints. Every abnormality of a certain degree is no good. In my opinion small inner toes should be concidered serious, specially if the toes are standing close together and there are abnormalities also in the pasterns.

In my opinion judgement of exterior conformation is of significance, provided one has satisfactory criteriae concerning function upon which to base the judgement. The criteriae for the distal parts of the legs I think are known. The back, the overline, the quarter and hams including the stifle joints should also be judged functionally, not as amounts of meat or fat. The possibility that feeding determined growth rate and environmental factors influence some exterior traits should be taken into conciderration if pigs are going to be ranked.

c) Joint shape

The etiology of osteochondrosis is complex. It is a generalized disease, but in my opinion local overloading in the joints is also a significant factor. Local overloading will occur in an unstable joint. Joint stability depends on muscles, ligaments and joint shape. Joint shape can be measured, and seems to be inherited to some degree, but the heritability has not jet been calculated. Own investigations of joint shape was carried out to throw light on the etiology of osteochondrosis, and if correlations were found, get the possibility to use joint shape (and exterior conformation) as selection criteriae. The work was concentrated on the elbow and the stifle joint. Generally said, elbow joints with distinct guiding ridges and with surfaces which were steep and formed large angles with other joint surfaces on the same bone and same joint, showed the least degree of lesions. A correlation coefficient for an index based on 6 different anatomical details and lesion score in the elbow was about 0,7. The shape of the joint seemed not to be influenced by the lesions. The elbow shape can not be judged in vivo, even by using x-ray, so an use of these criteriae in selection must be based on littermates or offspring tests.

The stifle joint has an overall unfavourable shape which leads to local overloading of the medial condyle of femur via the medial part of the intercondyloid eminence of tibia. Concerning the stifle joint it is not easy to say what is cause and what is effect when discussing joint lesions and special joint shape. In feeding determined rapid growing pigs there obviously was an affection of the endochondral ossification both distally and proximaly in the femur, resulting in altered joint shape. What is clear in my opinion, however, is that if pigs genetically has an unfavourable joint shape they are very prone to joint affection, and will easily get into an evil circle. A low, twisted medial condyle of femur is unfavourable. This condition might be seen in vivo as it might result in bow hind legs. A high lateral part of the intercondyloid eminence of tibia compared with the medial part is unfavourable, the same with thin poorly covering menisci. Both for the elbow and the stifle joints there was a relationship between well shaped joint and ability to move. Two main reasons for this seem to be logical. The cause might be the higher

degree of osteochondrosis in unfavourable shaped joints. It might also be the fact that a well shaped joint will be easier for the pig to "lead", and therefore give the pig a better gait.

d) "General strength"

There are results showing that the domestic pigs, whether they are fast-growing "modern", relatively "unmodern" or minipigs have some weak constitutional traits compared to other kinds of animals. Nevertheless, breed differences are also present, indicating that there also must be differences in general strength between pig populations. Investigations concerning general strength often deals with the skeleton. It seems to be clear that there is a discrepancy between growth rate and maturing rate in modern pigs, specially concerning the bones and cartilage. Probably it is general and also concerns the collagene of ligaments and so on. It is also known that there is variations in the muscle quality between kinds of animals and between breeds. Criteriae strongly correlated to general strength would give us an unestimable tool in the work for better constitution of animals. However, these criteriae seem not to be easy to obtain.

Prevention of leg weakness

We all know the complaints against the modern pigs constitution. It would be more correct to say the pigs condition, as it is this we observe. Whether the underlying causes for poor condition are mainly of feeding, environmental or herditary origin is not easy to tell for one single animal. Generally, however, it seems to be agreed that modern pig breeds are constitutional "weaker" than their old ancestors. If we demand a pig with the same condition as earlier, it therefore is reasonable to suppose that balanced feeding, environmental factors and exterior conformation are more important than before.

By giving functionally wellshaped pigs highly restricted feeding and a "good" environment it seems that the leg problems for those individuals would be small. However, this is on the one hand expensive, on the other most probable not a good solution in the long run, even though it could be done with the breeding pigs if the selection for backfat thickness etc. was carried out on littermates or offspring. Animals used only for production of slaughterpigs could also be prepared like this. By preparing the breeding animals in large scale, however, we might get too large leg probblems in the slaughterpigs after a while, as we then would have a very moderate "natural" selection. A practical test, that means to give the breeding animals the environment and feeding which the slaughterpigs have, must be an insurance of great value. According to geneticists, this is of special value if there is an interaction between constitution and environment/feeding. When using practical tests we do not know exactly what we are selecting for, but the sum of it is "the ability to stand modern feeding and environment".

The most dangerous selection when thinking about constitutional problems, is selection for one spesial characteristic, i.e. large hams or long back. An index combining several characteristics, i.e. also fertility measured as number of pigs at weaning, will be a more safe way. It seems to be difficult to get criteriae about leg weakness that at the moment can be used in an index. A selection against leg weakness at the moment must therefore probably be based on supplemental informations concerning i.e. movements and exterior conformation of the individuals as well as its relatives. Number and quality of nipples, temper, some deformities, shivering etc. are also characteristics that can be valuable supplemental informations in selection.

A prevention of leg weakness based on general constitutional improvement demands informations in relatively large scale. The low age of breeding animals at slaughter makes it difficult to get the informations in time. Average age of breeding boars in Norway are less than 1½ years, for sows about 2 to 2½ years. Boars in artificial insemination might be kept longer time, and deep freezing of boar semen will also open new possibilities in breeding. Another useful way is probably hybrid systems.

The fact that both the leg weakness problems and the selection systems are different in different countries makes it essential to get geneticists and veterinarians into team work, both to draw general lines and to solve the special local problems.

MACROSCOPICAL AND MICROSCOPICAL FEATURES IN PORCINE OSTEOCHONDROPATHIES

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For twenty-five years it had been generalised metabolic disorders in the porcine skeleton as rickets, osteomalacia, osteoporosis and osteodystrophia which were responsible for the osteochondropathies of that time.
Especially the last fifteen years it has been the more or less generalised alterations in the growthplates and the joint-cartilage with the underlying subchondral bone which in the slaughter pigs and breeding pigs have been the lesions which occur most.
About these lesions the macroscopically and microscopically visible pathological changes are shown by means of slides and the possible pathogenesis of these alterations will be discussed.
Pathological-anatomically these changes can be classified in two main groups:

Osteochondrosis
- changes in the epiphyseal plate and underlying metaphyseal bone
- changes in the enchondral ossification and the underlying subchondral bone of the joint cartilage

Arthrosis
- changes in the superficial, or the superficial and deep layers of the joint cartilage
- changes in the nucleus pulposus of the intervertebral discs.

The main difference between these groups is their location and the fact that the changes in osteochondrosis are only visible by sagithal incision of the joint cartilage and the bones, while the changes in arthrosis are well visible on regarding the joint cartilage without incisions. In both cases, however, it concerns aseptic predominantly functional mechanical changes. As regards the occurrence of the locations:

A. Epiphyseal plates
- distal ulna and radius
- proximal femur
- proximal humerus
- tuber ischii

Here it concerns the main locations. Most affected are the growth plates of the distal ulna, followed by the proximal femur, humerus, radius, ischium and the other here not mentioned epiphyseal plates.

B. Joints

stifle joint
- medial condyle of the distal femur

elbow joint
- medial and lateral condyle of the distal humerus
- semilunar notch

shoulder joint
- humeral caput
- acetabulum of the scapula

hip joint
- femoral caput

carpal joint
- articular surface of the distal radius
- distal carpal bones

hock joint
- articular surface of the distal tibia
- central and distal tarsal bones

vertebral column
- articular surfaces of the lumbar vertebrae

Most affected joints are the stifle joints, in some herds up to one hundred per cent of the pigs show lesions, in a lesser amount the elbow, shoulder, hip and the others, however, the latter still more than fifty per cent. In a joint there is mostly a typical area where the arthrotic and osteochondrotic lesions occur. In the stifle joint the medial condyle, and so on.
We will now give you a short survey of the macroscopically and microscopically changes in these osteochondrotic and arthrotic lesions on the different main locations.
In the distal ulna you can see macroscopically especially a local or diffuse irregular widened epiphyseal plate and changes in the metaphysis by an irregular ossification. Histopathologically there are predominantly regressive alterations in the cartilage as fissures, necrosis and demasking of the fibrils resulting in growth- and ossification disturbances. In the underlying metaphysis there are haemorrhages, osteoclasia and vascular changes. Besides there are progressive changes as cartilage proliferation, fibrosis and bone scelerosis. In the growth plates of the humeral and femoral head and tuber ischii there are similar changes. Besides you frequently find a local premature closure of the epiphyseal plates; in the proximal humerus towards the bicipital groove and in the femur towards the trochanter. The consequence is a flattening of the heads and a slipping down in a dorso lateral direction of these heads. This phenomenon occurs in different degrees. In some cases, often caused by sudden external traumatic factors, in these destructively changed epiphyseal plates it comes to a partial or total epiphysiolysis. This separation occurs most commonly at the head of the femur and at the tuber ischii and it is called epiphysiolysis

capitis femoris and apophysiolysis tuberis ischiadici. The first mentioned is found in older slaughter pigs and in breeding pigs, the second especially in younger breeding pigs. Microscopically the separation occurs in the area of enchondral ossification or in the area of resting cartilage. The changes in the joint cartilage and subchondral bone are mostly very typical in their locations and in their appearance in the different joints. In the head of the humerus and the femur there are mostly areas of atrophy, often without subchondral lesions, their appearance is reddish and their level is below the level of the surrounding cartilage. In the humerus their location is towards the bicipital groove and in the femur towards the drochanter. Histopathologically there is atrophia, a granular and vacuolar structure, demasking of the fibrils, brood-capsule formation of the chondrocytes and diminished ossification of the jointcartilage.

In the elbow joint the changes are especially in the lateral and medial condyles of the distal humerus, in the semilunar notch of the proximal ulna and in the articular surface of the proximal radius. In the condyles there are regions with invagination, defects and proliferation of the joint cartilage. In the semilunar notch the synovial groove is mostly very widened, deepened and irregular with loss and proliferation of cartilage. In the articular surface of the proximal radius there are defects and invaginations at a typical place. Microscopically there are regressive and proliferative changes in different degrees in the cartilage and subchondral bone.

In the articular surfaces of the distal radius and distal tibia there are especially invaginations of the joint cartilage, sometimes with defects. The carpal and tarsal bones show similar lesions, often the medial bones are involved more severely than the lateral bones. Histopathologically degenerative changes in the jointcartilage are predominant in all these bones.

Arthrosis deformans tarsi, a severe, often anchylosing arthrosis of the hockjoint, is compared with ten years ago, now of little significance.

Especially the stifle joint, the lateral areas of the medial condyles are involved. There is always flattening of the cartilage in this area with thickening of the cartilage and disturbance of the ossification. This stage is followed by frayed, fissured, invaginated, split, separated or folded cartilage which leads to defects and collapsed subchondral bone in this area. Besides there is proliferation of cartilage. The end stage is a very severe arthrosis deformans. Histologically there are regressive changes as vacuolar and fibrillar structure of the ground substance, degeneration of cartilage cells, atrophy, fissures and necrosis. The normal pattern of the cartilage cells is disturbed, brood-capsule formation of cartilage cells occur.

There are often large fissures in the osteochondral junction. In the underlying subchondral bone there are regressive and proliferative changes as fractures of trabeculae, haemorrhages, fibrosis, osteoclasia, loss of trabeculae, necrosis, osteosclerosis and vascular changes.

In the vertebral joints the lumber vertebral joints and lumbar intervertebral discs are mostly affected. In the discs the whole nucleus pulposus and the innerlayer of the annulus fribosus may be degenerated.

Concerning the possible pathogenesis of all these changes in the epiphyseal plates and in the jointcartilages the following rough outline can be followed. Regressive changes in the cartilage.

A. Disturbances in the diffusion of the groundsubstance:
   - granular and vacuolar structure
   - fibrillar structure

   resulting in fissures, necrosis, and defects.

B. Changes in the chondrocytes:
   - degeneration and necrosis
   - abnormal formation of groundsubstance

   resulting in: swelling
   fraying
   necrosis and defects
   - abnormal proliferation and maturation of the chondrocytes

   resulting in - atrophy and hypoplasia of the joint cartilage
   - insufficient subchondral ossification
   - flattening of the joint cartilage

C. Progressive changes in the cartilage.
   - regeneration of cartilage
   - brood-capsules formation
   - chondroblastema formation

D. Progressive and regressive changes in the subchondral bone.
   - fractures of spicules of caleified cartilage and trabeculae of bone.

   resulting in - myelofibrosis
   - osteoclasia
   - collapsing of the overlying joint cartilage
   - increased new bone formation (osteosclerosis)
   - vascular changes

   resulting in : aseptic necrosis of marrow and bone.

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BREEDING ASPECTS OF LEG WEAKNESS IN PIGS

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The major concern of this paper is breeding aspects. It therefore seems unnecessary to explain the symptoms of these diseases in detail, since the pathologists at this meeting are more competent to do so. It should, however, be quite useful to discuss the probable reasons and possibilities of minimizing the resulting losses. This should at the same time be an attempt to establish an urgently needed connection between animal breeding and husbandry on one hand, and pathology and clinical medicine on the other hand.

The widely differing terminology and diagnosis used in each discipline presents a crucial problem, which needs to be solved. Using our own results, I shall try to demonstrate this quite clearly.

In cooperation, our institute and the Institute for Veterinary pathology of the FU Berlin examined after slaughter the pathology of pigs, which had been part of a trial in Trenthorst. In these pigs, three significant deficiencies in the femur region could be noted: 1) changes in the epiphysial cartilage of the femoral head, 2) deformations of the medial condyle, and 3) microfractures of the metaphysial corticalis.

Considering the frequency of those deformations in pigs of various breeds - stated in Table 1 - large breed-dependent differences can be seen (Dämmrich und Unshelm, 1972; Unshelm et al., 1972). Rapid weight gain, achieved by breeding and feeding intensity, is the

Table 1. Frequency of deficiencies in the femur of 205-day-old pigs

Breed	n	Liveweight (kg)	Deficiencies in %		
			Condylus medialis	Metaphysial corticalis	Epiphyseal cartilage
German landrace	43	103	60	28	95
Piétrain	34	88	15	15	53
German pasture pig	50	112	18	74	60
Mangalica	27	79	7	74	37
Göttinger miniature pig	40	38	0	8	12

initiating factor for the frequently occurring changes in the epiphysial cartilage. Most deformations in the German Landrace pig are certainly associated with an inherited susceptibility for a more medially protruding femoral head. Rapid weight gain also causes deformations in the apparently inadequate articular cartilage of the medial condyle of the femur. This occurs more frequently in German Landrace pigs as a result of stress on the medial side of the extremities of this breed. The appearance of microfractures in the metaphysical bone cortex also shows a special susceptibility of this breed, which probably became clearly visible through feeding conditions used. All the pigs examined, were fed ad libitum, which - especially in breeds with low muscle building capacity, caused a high increase in live weight by fat development. The microfractures of the metaphysial corticalis which are frequently observed in Mangalica pigs and German pasture pigs, could thus be caused by the poor development of the cortex in these breeds in relation to their body weight.

This obvious connection of skeletal deformations and live weight led to a closer examination. For this study 72 female pigs (German Landrace), were divided into 2 groups of litter sisters. One group was fed restrictively, while the other group was provided with a highly concentrated ration ad libitum, with the result, that at slaughter, at the age of 205 days, the body weights were 72 and 125 kg respectively. Consequently, there was a nutritionally induced weight difference of 53 kg in pigs of same age and genetic background. The results with regard to localization and grade of deformations in the joints of fore and hind limbs show clearly, that damages in the skeleton in form of local growth disturbances caused by stress are found in both groups, but the extremely well fed (ad libitum) 53 kg heavier animals were damaged much more severely (Dämmrich und Unshelm, 1975).

Subsequently, breed and body weight are of great importance for the development of skeletal deficiencies. It was also found that virtually 100 % of the German Landrace pigs had microscopic defects in almost all joints. This figure strikingly differs to those observed in practice. The German litter testing stations recorded leg weakness in 8 - 18 % of the animals of which close to 2 % were total failures. This means, that only 8 - 18 % of the animals with histologically manifested defects displayed also visible symptoms of leg weakness, a classification which does not even indicate the side effected.

The problem facing animal breeding and husbandry consists plainly in finding appropriate measures for counteracting leg weakness. Of the many factors described as causetive for leg weakness, the most prominent one is brought about by breeding and

feeding, namely the dissociation of weight increment and maturity which puts too much weight on an immature and therefore too fragile skeleton. This has been demonstrated by the results of the previously mentioned investigations on pigs of the same age both from various breeds and with extremely different rations. Since economical aspects do not allow for a decrease in weight increments, means have to be found to increase skeleton stability. None of any practicability are available at presence. The question for the chances of succeeding is identical with that for the degree of heritability of the skeleton defects, but there again difficulties in interpretation arise. The h^2-values estimated for the unfortunately vague characteristic leg weakness will be in the 0,18 - 0,22 range (Pfleiderer, 1973). This would allow for a systematic selection, but it has to be considered that this figure accounts only for the undoubtedly visible deficiencies that, on the same material, a significantly positive correlation between carcass quality and leg defects was evident. Breeding based on leg weakness observations would therefore only take care of part of the overall defects. In addition the danger that such a breeding measure would lastly result in a decreased performance is imminent.

Other measures for decreasing the occurrence of leg defects may arise from the now highly actual hybrid-breeding. We were engaged in rather extensive experiments of this type, but the results are unfortunately not yet available.

With respect to the husbandry, everything enhancing the occurrence of leg defects must of course be avoided. This is the case for systems using fully iron grated floors which cause significantly higher losses. Costly prophylactic measures against leg defects, which have been tried experimentially, mostly in broiler production, but also with pigs, must for economical reasons be restricted to valuable breeding stock.

Thus, the hope remains that animal breeding and animal husbandry, the pathologists, and the clinicians, and all associations involved, coordinate their efforts for a successful cooperation or it will not be possible to solve the problem of the so-called leg weakness.

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VASCULAR LESIONS IN EPIPHYSIOLYSIS CAPITIS FEMORIS IN SWINE

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Summary

Uptill now research into the blood supply of the femoral head has mainly been a histological one. Decreases in the size of the lumen of large as well as small arteries have been found. Very often it has been impossible to say if the changes found were original alterations or just secundary findings. In order to obtain more information about possible vascular abnormalities, vascular perfusion was carried out in one healthy and three affected animals. A clear arteriogram was seen in the femur of the healthy pig. In the diseased ones no vascular perfusion was seen in the femoral head, since the contact with the vessels in the periost already had been interrupted. The small arteries in the metaphysis were filled rather poorly, while in the large arteries strictures were seen. The occurence of these strictures is looked upon as being of importance, since their occurence is difficult to explain from malformation of the femoral head, mechanical pressure and so on. Another somewhat peculiar finding was the place of the fracture between the bone and the cartilage at the side of the epiphysis.

Introduction

In the Netherlands epiphysiolysis capitis femoris is seen in both young breeding animals and fatteners over 50 kg bodyweight. The etiology of epiphysiolysis is not known exactly. As the most likely and important factors are mentioned: variation in shape of the femoral head; a disproportion between the mechanical load, bodyweight and the resistance of cartilage and bone tissue; rapidity of weight gain and total weight with respect to the maturation of the skeleton. Environmental factors too are of importance.

The influence of the Dutch Landrace is well known (Schilling, 1963). The use of this pig and similar breeds in various breeding programs resulted in a change of type of pig. In breeding programs of pure breeds too the more meaty type of animal was selected. This caused an increase in the length of the animals and a change in statics and dynamics between the spinal column and the limbs. The length of the bones in the limbs too increased (Dämmrich et al., 1972). The position of the femoral head with respect to the femur itself changed too. This causes a change in the point of impact of pressure on the femoral head as can be seen in the direction of the cartilage columns (Dämmrich et al., 1972; Grøndalen, 1974).

Vascular abnormalities have been described in the epiphysial and the metaphysial side near the growth plate (Thurley, 1969; Herrmann, 1972). The results of vascular lesions have been studied experimentally by inducing pressure (Trueta et al., 1960; Trueta et al., 1961). The majority of the abnormalities was seen at the metaphysial side. The blood vessels at this side seem to be less protected against pressure as those at the epiphysial side. The changes in the blood vessels result in a decrease of the lumen. The histological changes seen are disturbances in growth and ossification of the cartilage and the primary and secundary spongiosa underneath, fissures and necrosis. Besides these regressive changes more progressive changes can be found in older cases: cartilage cell proliferation and intensified bone formation.

Material and Methods

One healthy and three diseased animals were used for vascular perfusion. The healthy one, a gilt of 5 months old, had never shown any symptoms that might indicate epiphysiolysis or clinical osteochondrosis. X-ray did not show any abnormalities in the hip joint. At autopsy only minor symptoms of osteochondrosis were found.

The diseased animals ranged in age from 4½ to just over 6 months of age. Two were female, one a castrated male. They all showed symptoms of acute epiphysiolysis: reluctance to stand up and to move, abnormal gait, strong pain reactions to passive movement of the hip joint and crepitation could be felt and heard. X-ray confirmed the diagnosis.

Vascular perfusion was carried out within three days of the onset of the symptoms. After the intravenous administration of 25000 I.U. of heparine and a general anaesthetic the animals were bled. The abdomen was opened, the intestines pushed aside and the aorta and posterior vena cava were exposed. Both vessels were ligated to the anterior side, infusion tubes were put in both vessels directed caudally. Via the aorta 3 l. of physiological saline solution was used to wash out whatever blood was left in circulatory system of the hind limbs. After this 300 g. of $BaSO_4$ dissolved in 800 cc of a physiological saline solution was given till the $BaSO_4$ appeared from the vena cava. Then a solution of 300 g. $BaSO_4$ in 800 cc 4% neutral formalin was allowed 18 hours to infuse. Autopsy was carried out and both femurs were taken out and cleaned of all muscle and tendon tissue up to the periosteum. They were X-rayed and examined histologically without decalcification. The sections were coloured according to the Goldner method.

Results and Discussion

In both the normal femurs the three blood vessel systems, which supply blood to the growth plate area, can be seen very clearly:
a. small, well filled arteries enter the epiphysis from the periosteum just above the growth plate;
b. from the periosteum similar small arteries enter the metaphysis;
c. large arteries enter the metaphysis from the diaphysis.

The large arteries are well filled all through their course to the metaphysis. An equal filling is also seen in the small arteries around the growth plate. At the X-rays osteoporosis in the bone is not seen.

In the diseased animals filled blood vessels are not seen in the epiphysis. Although the seperation of the femoral head was not yet complete, the damage to the periosteum was already so severe that the continuity of the vessels between the periosteum and the epiphysis had been interrupted.

Much more important are the various strictures seen in the large arteries in the diaphysis. These strictures can also be found in the small arteries underneath the growth plate. Compared with those in the normal femurs the arteries in the abnormal femurs towards the metaphysis are filled rather badly. The X-rays show osteoporosis in the majority of the abnormal femurs.

Compared with arteries in the normal femurs the arteries in the abnormal ones showed a large number of alteration, especially in the small arteries. Alterations found were: fibrosis, hypertrofia and proliferation of the media; proliferation of the intima; a frayed elastic internal coat and a fibrin blockade of the decreased lumen of the arteries.

The site of the fracture was at the epiphysial side between the bone and the cartilage.

Although a number of alterations have been found in both the vascular perfusion and the histological examinations, it still is difficult to estimate their proper value. The alterations in the small arteries in the metaphysis can be caused by high pressure together with the malformation of the femoral head. The insufficient blood supply caused by these alterations contributes to the degree of the dystrofic proces in the growth plate. The occurence of strictures in the large arteries in the diaphysis however is very difficult to explain with just the wrong course of the pressure lines and an abnormal shape of the femoral head. The effect of these strictures is very clear. They cause a decrease in the blood supply to the small arteries in the metaphysis and by doing so increase the effect of the alterations in these arteries and their role in the development of epiphysiolysis capitis femoris.

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SOME ASPECTS OF THE (PATHO)MORPHOLOGY OF THE GENITAL ORGANS IN GILTS

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The investigation concerned the genital organs of gilts (Dutch Landrace, Dutch Large White and Dutch Landrace x Dutch Large White),which were slaughtered in connection with a food-experiment. The age of the gilts was 8-9 months.The examined gilts can be classified in 3 groups (Table 1).

Table 1. Classification of the examined gilts

Group	Number of gilts
A. Delayed puberty or anoestrus	31
B. Sexually mature, non-pregnant after one artificial insemination	32
C. Pregnant (25-31 days)	51

Most attention will be paid to the groups A. and B. Of course these two groups are very important because the fertility in swine is of great economic importance.
Ad group A:
It is very difficult to determine the age from which it is allowed to use the term "delayed puberty" or "anoestrus". This age is not only dependent on the breed, but there are also great individual differences. Moreover this age is dependent on external circumstances. According to the literature about this subject this age is about seven months. Assuming the latter age to be right, it seems to be correct to speak of "delayed puberty" or "anoestrus" in our cases.
Ad group B:
The pigs of this group are inseminated during the first, the second or the third oestrus. One could at least speak of subfertility with regard to this group as a whole. However, the individual pigs in this group might be fertile, subfertile or infertile.

The following subjects are discussed:
I The morphology of the vaginal epithelium (in the groups B and C) and the cervical epithelium (in all 3 groups).
II The functional state of the ovaries of group A.
III The weights of the uteri (exclusive of the cervix) and the lengths of the uterine horns of group A, as compared with those of group B.
IV Some pathomorphological findings (in all 3 groups):
1. Segmental aplasia of the uterus in group B.
2. Parovarian cysts in all 3 groups
3. Inflammation of the cervix and/or the vagina in all 3 groups.

I The morphology of the vagina and the cervical epithelium.

1. The vaginal epithelium (the anterior vagina).

The morphology of the vaginal epithelium is a.o. important in pregnancy diagnosis. The most striking changes concern the number of cell layers. According to literature the epithelium consists of 10-20 cell layers during the oestrus. Towards the di-oestrus this number decreases to 4 or 3 cell layers.
From some days before the beginning of the following oestrus the number of cell layers increases again. All the pigs of group B were slaughtered within 7 days after the beginning of the oestrus. Therefore we were not able to study all the cyclic changes. Our findings within these 7 days are in agreement with the literature.
In pregnant pigs the number of cell layers is only 2 or 3. During our investigation of the 51 pregnant pigs (see Table 1, group C.),it appeared that except for 2 vaginae, the number of cell layers was indeed only 2 or 3. The epithelium of these 2 vaginae consisted of more than 3 cell layers.The number of cell layers is not the only difference in vaginal histology between pregnant and non-pregnant pigs.

2. The cervical epithelium

The cervix consists of 2 parts:

The caudal part = ectocervix and the cranial part = endocervix.
The ectocervix is covered with squamous epithelium and the endocervix with cylindric epithelium. Below the cylindric epithelium of the endocervix in man groups or rows of cells can be found, which are characterized by only scanty cytoplasm and a relatively large nucleus. These cells show a very uniform aspect. They are called reserve cells or subcolumnar or subcylindric cells. In our material we regularly saw in all 3 groups cells which, at any rate lightmicroscopically, correspond with the before mentioned reserve cells. Regularly we also found in all 3 groups reserve cell hyperplasia with the same morphology as in human cervices. In man these reserve cell hyperplasia could develop in cases of inflammation of the cervix and also under the influence of hormones, especially of oestrogens. In man the occurrence of reserve cell hyperplasia need not be abnormal. In our material we saw the reserve cell hyperplasia both in cases of cervicitis and in cervices without inflammation. Besides on the analogy of the situation in man the occurrence of the reserve cell hyperplasia in pigs might be normal. In man the reserve cells of the cervix have been studied well, especially in connection with the pathogenesis of cervix tumors. These tumors could develop from reserve cells. In view of the occurrence of reserve cells and of reserve cell hyperplasia in the cervix in pigs with, at any rate lightmicroscopically, the same morphology as in man, the pig seems to be a good experimental animal for comparative research concerning the biological and the pathobiological behaviour of these cells.

II The functional state of the ovaries of group A.

The ovaries of group A did not contain any corpora lutea nor any fully developed follicles either. This finding is an indication that these pigs which did not show external oestrus symptoms did not ovulate either.

III. The weights of the uteri (exclusive of the cervix) and the lengths of the uterine horns in group A, as compared with those of group B.

Except for one, the weights of the uteri of group A were apparently lower than those of group B (Fig.1) and except for a few the uterine horns in group A were also apparently shorter than those in group B (Fig.2). Uterus a of Fig. 1 is the same as uterus a of Fig. 2.

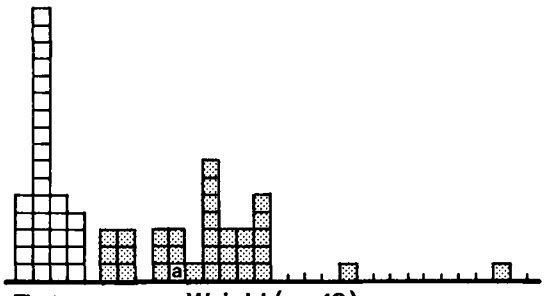

Fig.1 Weight (g x 10)

Fig.2 Length (cm)

☐ Prepuberal or anoestrous. Number 31
▨ Sexually mature (nullipar). Number 32

In view of the observations mentioned under the pointsII and III it can be concluded that the uteri of group A show the picture of juvenile uteri.

IV Some pathomorphological findings

1. In group B a segmental aplasia of the uterus was found once. At the corpus side a part of the right horn was absent. Segmental aplasia of the uterus is a well known condition in many species.
2. In the groups A and B we regularly found parovarian cysts with a maximal diameter of 4 cm. Presumably these cysts arise from persisting embryonal structures (the mesonephros, the Wolffian duct and the Müllerian duct).
Probably these cysts have nothing to do with the "delayed puberty" (group A) and it also remains to be seen whether they are of causal importance concerning the subfertility (group B). In the group of pregnant pigs these cysts were also regularly found.
3. In some cases in the groups A and B inflammation of the cervix and/or the vagina was found, a little more often than in the group of pregnant pigs. The significance of the above mentioned inflammation is not clear.
In most cases the macroscopical and the lightmicroscopical examination of the genital organs did not reveal the explanation of the "delayed puberty" or "anoestrus" (group A) and the subferti-

lity (group B) respectively. In many cases no morphological changes were found and if they were found, it was the question whether they were of causal importance (e.g. the parovarian cysts and the inflammation of the cervix and/or the vagina). It may be possible that the cause can be found in the hypothalamus-hypopysis system, especially in the case of the "delayed puberty" or "anoestrus". Moreover it remains a matter of discussion whether or not it is abnormal when 8-9 months old pigs are not yet sexually mature. Anyhow looked at economically it is an undesirable situation.

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