AN INVESTIGATION INTO THE EXTENT TO WHICH VARIOUS DIETARY COMPONENTS, PARTICULARLY LACTOSE, ARE RELATED TO THE INCIDENCE OF DIARRHOEA IN MILK-FED CALVES

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Proefschrift
ter verkrijging van de graad
van doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op vrijdag 21 november 1980
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen.

H. VEENMAN & ZONEN B.V.–WAGENINGEN–1980

STELLINGEN

I

Ten onrechte veronderstelt ROY een verband tussen diarree bij kalveren en de eiwitfractie van de opgenomen kunstmelk.

ROY, J. H. B. (1970). The calf, Vol. 2, p. 124. Iliffe Book Ltd., London.
Dit proefschrift.

II

De ruwvoeropname van melkkoeien wordt niet beïnvloed door het ruwvoeraandeel in het rantsoen gedurende hun opfok.

BALCH, C. C. et al. (1960). Br. J. Nutr. **14**: 379.

III

De individuele verschillen in ruwvoeropname van jongvee geven geen inzicht in de verschillen in ruwvoeropname op oudere leeftijd.

IV

De schijnbare verteerbaarheid van aminozuren bij varkens dient aan het einde van het ileum te worden vastgesteld.

ZEBROWSKA, T. (1978). Feedstuffs, **25** december.
LENIS, N. P. (1980). Rapport no. **133** I.V.V.O., Lelystad.
Dit proefschrift.

V

Het gereglementeerd gebruik van antibiotica in veevoeders ter stimulering van de groei is minder gevaarlijk dan het gebruik van koper voor dit doel.

WILLIAM SMITH, H. (1969). Vet. Rec. **85**: 31.
SOLOMONS, I. A. (1978). J. An. Sc. **46**: 1360.
HIRCH, D. C. et al. (1978). J. An. Sc. **46**: 1425.
Rapport Werkgroep 'Mineralen in krachtvoer in relatie tot bemesting en milieu' NRLO, 1979.

VI

De mogelijkheden van rietzwenkgras *(Festuca arundinacea Schreb)* in de graslandproduktie verdienen in ons land meer aandacht.

LUTEN, W. (1977). Bedr. Ontw. **8**: 435.

VII

Bij het afwegen van de vóór- en nádelen van een hoge rundveebezetting wordt onvoldoende rekening gehouden met de consequenties voor de graszodekwaliteit.

VIII

In goed overleg tussen onderzoek, voorlichting en industrie zullen op korte termijn bindende afspraken moeten worden gemaakt over de veevoedingstechnische kennis, die in de 'software' van automatiseringstechnieken in de rundveehouderij gebruikt gaat worden.

IX

Zomerstalvoedering bij melkvee is zowel uit ecologisch en landschappelijk oogpunt, als op grond van dierziektepreventie af te wijzen.

X

Het 'Wetenschap & Samenleving' onderwijs moet in de eindfase van de studie worden gegeven.

XI

De landbouwhuisdieren, de veehouders, noch de consumenten van dierlijke voedingsmiddelen zijn gebaat met dogmatische ideologen, wél met inventieve idealisten.

XII

Friezen en Groningers verschillen boven Zwolle significant.

Proefschrift van G. Hof
An investigation into the extent to which various dietary components, particularly lactose, are related to the incidence of diarrhoea in milk-fed calves.

Wageningen, 21 november 1980.

VOORWOORD

De afsluiting van dit onderzoek met een proefschrift leidt onvermijdelijk naar een terugblik op de afgelopen jaren, waarin velen hebben meegewerkt om dit resultaat te bereiken. Het zou te ver voeren allen hier persoonlijk te bedanken, maar een aantal wil ik graag met name noemen.

Hooggeleerde Boer Iwema, hooggeachte promotor, mijn dank richt zich vooral op de grote vrijheid die ik kreeg bij het vaststellen van het onderwerp van onderzoek en nadien bij de uitvoering. Dit vertrouwen heeft mij meer gestimuleerd, dan U wellicht zult vermoeden.

Hooggeleerde Van 't Klooster, hooggeachte promotor, Uw overtuiging dat de resultaten van dit onderzoek zich leenden voor een promotie is de stimulans geweest voor dit proefschrift. Uw kritische opmerkingen over dit, ook voor U, niet alledaagse onderwerp leidden niet alleen tot prettige en leerzame discussies, maar bewezen tevens dat U zich veel tijd en moeite heeft willen getroosten om onze interpretaties kritisch te evalueren.

Een groot aantal medewerkers van het ILOB heeft zich ingespannen de proeven nauwgezet uit te voeren, ondanks de vele nachtelijke en weekend-uren die hiervoor moesten worden opgeofferd. Het heeft mij bijzonder getroffen, dat deze gezamenlijke inspanning geleid heeft tot uitstekende, persoonlijke verhoudingen. Met name wil ik D. van Kleef en P. van Leeuwen danken, die met hun inzet borg stonden voor een uitstekende verzorging van de proefdieren, een nauwgezette uitvoering van het onderzoek en een goede begeleiding van meewerkende studenten. In één adem moet hierbij de analytische afdeling worden vermeld, die onder leiding van W.Onk er altijd in slaagde het grote aantal monsters tijdig te analyseren. Vooral jullie inspanning de koolhydraten-analyses tot bruikbare resultaten te vertalen verdient alle lof. Niet in de laatste plaats wil ik K. van Hellemond, J. Huisman en E. van Weerden bedanken voor hun bemoeienissen met de organisatie van het onderzoek en voor hun kritische evaluatie van proefopzetten, -uitkomsten en interpretatie. Dat het onderzoek tot dit proefschrift heeft geleid is mede te danken aan jullie inspanning.

Mr. C.A. Shacklady was zo vriendelijk de engelse tekst te corrigeren. Daarnaast heeft zijn kennis van het vakgebied mij bijzonder geholpen om 'sence' en 'nonsense' scherp te onderscheiden bij het opstellen van de uiteindelijke tekst.

De grafieken werden getekend door W. Heije. Het feit, dat uiteindelijk slechts een klein deel van de tekeningen in dit proefschrift zijn opgenomen, doet niets af aan de kwaliteit van zijn werk.

De afdeling tekstverwerking heeft zich bijzonder ingespannen om het typewerk nauwgezet en op tijd te verzorgen. Ik heb grote bewondering voor de wijze, waarop men steeds opnieuw bereid was correcties, wijzigingen en aanvullingen aan te brengen. Tot slot wil ik niet nalaten P. J. Lenaers te bedanken voor zijn bijdrage. Hoewel je slechts gedeeltelijk was betrokken bij dit onderzoek, is je bijdrage van grote waarde geweest. Het feit, dat je zelfstandig een groot aantal andere werkzaamheden voor je rekening nam was een essentieel onderdeel van, en een geruststellende gedachte bij de samenstelling van dit proefschrift.

CONTENTS

1. GENERAL INTRODUCTION

Diarrhoea follows on a serious disturbance of normal gut function in young calves. Improved veterinary control and treatment, as well as the improvement of management and nutrition have reduced considerably the mortality rate. Nevertheless scouring is still the most important disturbance in the new-born calf and is a perennial problem to the farmer, the veterinarian and the scientist engaged in calf research. But the greatest sufferer is the animal itself.

In the literature on diarrhoea usually a clear distinction is made between scouring resulting from pathogenic infections and that caused by nutritional factors. This distinction, however, does not exclude interrelationships between the two. Gut digesta serve directly or indirectly as the substrate for the growth and multiplication of pathogenic organisms. The quantity and composition of intestinal digesta may therefore constitute a predisposing factor in the development of pathogenic organisms.

In practice it is well-known that the new-born calf is highly susceptible to diarrhoea of either nutritional or pathogenic origin. Several precautions have to be taken in the nutrition and management of these young animals to prevent the derangement as much as possible. The nutritional precautions start by feeding colostrum as early as possible after birth. Thereafter it is still necessary to control the daily feed intake and frequency of feeding as well as the time required to change to a different dietary regime. These precautions reflect the limited capacity of young calves to digest milk diets.

In commercial veal calf production milk intake is designed to achieve maximum growth rate. Therefore the daily feed intake is usually only slightly below the tolerable limit. It must be stressed that these requirements of milk-fed calves impose severe restrictions upon the type of feed ingredients that are suitable for use in milk replacers.

The farmer frequently uses faecal consistency as an indication for the calf's tolerance of the diet, the consistency becoming loose when the limit of tolerance is reached and diarrhoeic when this limit is exceeded. The small margin that exists between normal physiological function of the digestive processes and digestive disorders justified, in our view, an investigation into the relationship between dietary factors and the incidence of scours in milk-fed calves.

The object of this work was to become better informed about the effect of excessive intakes of dietary components on digestive processes. It included experiments where the main objective was to classify common diet components according to their diarrhoeic properties in milk-fed calves. In subsequent experiments the related changes in digesta composition, flow rate and transit time, as well as component digestion and absorption were measured. Associated changes in bacterial population in the lower intestine and in the electrolyte and water metabolism in body fluids were not investigated.

2. DIET COMPOSITION AND FEEDING LEVEL IN RELATION TO DIARRHOEA. SURVEY OF THE LITERATURE

2.1. INTRODUCTION

Diarrhoea is basically a departure from the normal water and electrolyte metabolism in the intestine, resulting in an enhanced water excretion in the faeces. Several factors may be involved in this phenomenon; among these are:
- interruption of the transport processes across the mucosal cell membranes,
- high amounts of osmotically active substances in the lower gut lumen,
- abnormal intestinal motility.

In the literature the second factor is usually considered as that mainly responsible for nutritionally induced diarrhoea in healthy calves. Therefore particular attention is paid to the osmotic aspects of diarrhoea in this chapter. The literature on the relationships between digesta osmolality and scours in milk-fed calves is rather scarce. More information is available from research in man. This has been extensively reviewed by FORDTRAN (1967) and more recently by PHILLIPS (1972) and MICHELL (1974). The bacterial flora in the lower intestine may participate in osmotic diarrhoea. With regard to these mechanisms the literature usually refers to the classic work of WEIJERS et al. (1965), who reviewed these aspects in infants. Only some general aspects of the role of the micro-organisms will be considered. Finally attention is drawn to the function of osmosis as a factor in diarrhoea in calves.

2.2. THE OSMOTIC EFFECTS IN DIARRHOEA

Under normal physiological conditions water absorption and secretion in the intestine is a passive process, depending on differences in osmolality between the serosal and lumen side of the mucosa. This difference always exists when feed is digested and absorbed.

The components responsible for digesta osmolality are primarily inorganic solutes, of which sodium is the major cation at least in the lower part of the intestine, and soluble organic components, like amino acids, free fatty acids (FFA), mono- and to a lesser extent oligosaccharides. Other dietary compounds in the digesta contribute little to digesta osmosis in the small intestine. After enzymatic digestion they may exhibit some osmotic activity in the intestinal lumen. However, the end products of enzymatic digestion are readily and mostly actively absorbed by calves in healthy condition and when practical diets are fed. The attempts to attain isosmolality at the lumen and serosal site of the mucosal wall result in a simultaneous water transfer with the absorption of these solutes. Microbial fermentation of unabsorbed organic components will only in the large

intestine significantly interfere in osmosis (see section 2.4).

The soluble electrolytes are thus primarily responsible for the osmolality in the lumen of the small gut. According to current views, inorganic cations are partly passively absorbed in this section of the intestine (FORDTRAN, 1967; VAN 'T KLOOSTER, 1967). They pass the mucosal wall with the water flow ('solvent drag process'). They also may be absorbed against an electro-chemical gradient, due to the active absorption of other electrolytes, as is e.g. demonstrated with potassium (PHILLIPS, 1972). Sodium is primarily absorbed by an active transport mechanism and is furtheron coupled to the active absorption of monosaccharides and amino acids. The movement of anions in the small intestine is less well understood. Chlorine absorption and secretion seem to be related to bicarbonate movement and probably to the transport of sodium and hydrogen ion exchange (TURNBERG et al., 1970).

In the large intestine considerable absorption of sodium has been reported by SMITH (1962) and MYLREA (1966) in calves, and by VAN WEERDEN (1961); VAN 'T KLOOSTER (1967) and ROGERS et al. (1969) in adult cows. It is generally accepted that this is an active absorption process, possibly partly to balance the excretion of bicarbonate into the lumen. Potassium is passively absorbed or secreted in the colon. Normally the large intestine absorbs potassium, but in diarrhoea excretion may occur (PHILLIPS, 1972; FISHER, 1972, 1978). The water absorption in the hind gut seems generally to be related to sodium absorption. As this cation is the main osmotically active component absorbed in the large intestine, water absorption is presumably secondary to the absorption of soluble components in that section.

The movement of the solutes during the digestion of the feed quickly results in about isosmolality of the digesta with bloodplasma. Diets exerting low osmotic activity after ingestion are rendered isotonic by excretion of ions, in particular sodium and chlorine, and simultaneous water absorption. Conversely, in the case of diets containing high amounts of soluble components, isosmolality is reached by transfer of water from the mucosal cells into the gut lumen and rapid absorption of ions and organic solutes. Several authors presumed that under normal physiological conditions isosmolality is achieved before the digesta leave the duodenum.

Abnormalities in water absorption, as is observed in nutritional diarrhoea, are hence caused by insufficient absorption of osmotically active solutes from intestinal digesta. Excessive intake of feed (components) may go beyond the digestive and/or absorptive capacity of the small gut, as well as that of the colon. Their osmotic effects will simultaneously reduce the water absorption (39, 54, 57, 151, 152). In that condition water and electrolyte excretion with the faeces will increase (50, 51, 53, 54, 57, 117). Extensive losses of electrolytes may finally result in lower concentration of these solutes in the blood serum (10, 18, 28, 39, 54). This may affect the acid-base equilibrium in body fluids, resulting in acidosis, which is frequently found in calves suffering from severe scours (MITCHELL, 1974; DALLENGA, 1976; FISHER et al., 1978).

2.3. The role of the intestinal bacterial flora in nutritional diarrhoea

The bacterial population in the ileo-caecal region and particularly in the colon may respond to changes in digesta composition. In healthy, adult animals Bacteroïdes species dominate in that site because of their high efficiency in using the available substrate (Clarke et al., 1977). The literature is, however, less documented whether or not these species also dominate in the normal bacterial flora in milk-fed calves. The literature on these animals usually refers to the work of Weijers et al. (1965). These authors distinguished in infant colonic lumen two other groups of micro-organisms; the saccharolytic and the saccharo-proteolytic bacteria. The saccharolytic bacteria, mainly consisting of Lactobacilleae, use carbohydrates as a substrate, which are fermented into organic acids of low molecular weight, in particular lactic acid.

Among the saccharo-proteolytic bacteria several Enterobacteriaceae species, including E. Coli, and Bacillaceae species, like Clostridiae, can be distinguished. The substrate of these bacteria does not consist only of carbohydrates but they can also split N-containing compounds like proteins and peptides. The main end products of the proteolytic fermentation are ammonia and amines. It is generally accepted that these two groups of bacteria also exist in the colonic chyme of milk-fed calves, although their respective species may differ to some extent from those observed in infants (Roy, 1964, 1969).

In normal feeding conditions a balance exists between the saccharolytic and saccharo-proteolytic bacteria. However, the growth and multiplication of the saccharolytic flora in particular is stimulated when higher amounts of carbohydrates become available in the colon. The enhanced saccharolytic fermentation results in a higher production of fermentation end products with osmotic activity. When the absorption rate of the end products remains lower than their rate of production in the hind gut, isosmosis is achieved by reduced water absorption, and fermentative diarrhoea will occur. This type of diarrhoea is characterized by a low faecal pH, because of the high amounts of organic acids excreted.

Multiplication of the saccharo-proteolytic flora is generally supposed to occur, when high amounts of peptides and/or proteins are present in colonic chyme. Their fermentation may also induce osmotic diarrhoea. In that condition scouring is usually associated with a high faecal pH because of the high ammonia content.

As well as their effect in osmosis, the end products of fermentation may exert direct toxic effects on the intestinal mucosa. It is not clearly understood, whether or not the end products of saccharolytic fermentation possess toxic properties, directly promoting diarrhoea. In the literature toxic characteristics are usually attributed to high concentrations of lactic acid, because of its stimulating effect on the peristaltic movements of the intestine. Bennet et al. (1976) observed increased peristaltic movements, when infusing lactic acid interluminal in guinea pigs. Sodium lactate, however, seemed to induce an opposite response in these experiments. The excretion of both, lactic acid and sodium, is enhanced in

fermentative diarrhoea, as will be shown in Chpt. 5. The part played by lactic acid in this disturbance is therefore not yet clear.

According WEIJERS et al. (1965) and ROY (1969) the end products of proteolytic fermentation may irritate the mucosal wall and even cause lesions in the mucosal cells. They thus exert a direct toxic influence on the intestinal wall. As a consequence putrefactive diarrhoea can be more dangerous to the animal, particularly because this condition may become self-perpetuating. In such a condition even a change to a N-free diet will not stop the severe scouring. Nitrogen, released from the lesions, will then be used to meet the bacterial protein requirement.

2.4. FACTORS IN MILK SUBSTITUTE DIETS THAT PREDISPOSE CALVES TO DIARRHOEA

The significance of functioning of the digestive and absorptive processes in scouring clearly shows that the adjustment of diet composition and level of intake to the digestive ability of the young calves is most important. As the digestive function is only partly developed in these young animals, it puts strickt limits upon the type of components suitable for milk replacer diets.

In this survey on scouring no attention will be paid to the favourable aspects of the diet in the prevention of scours, although they are very important. In this context the benefits of colostrum in calf nutrition must be recognized, and in particular the importance of its immuno-globulins in the prevention of diarrhoea (KRUSE, 1970[a, b, c]). From the time the animals are changed over from the colostrum diet to whole milk or milk replacer, its quality and the feeding level become decisive for animal performance.

The diarrhoeic properties of milk substitute diets are less adequately treated in the literature than are the favourable ones. Although reference is often made to their existence.

Minerals

When the mineral intake exceeds the absorptive ability in the small intestine considerably, a higher amount will flow into the colon. The superfluous amount of electrolytes in colonic digesta may reduce the water absorption, because of their osmotic activity. Osmotic diarrhoea may occur in that condition (27, 134, 136, 181). The mineral content in milk replacers is consequently restricted, as is the inclusion of dietary components containing considerable amounts of soluble electrolytes, e.g. de-lactosed whey powder.

Protein

The effect of dietary protein intake on the incidence of scours has been extensively investigated, in particular by ROY and coworkers (150, 151, 152, 154, 155, 156, 157, 158, 159, 170, 171, 172, 173, 174, 175, 176). According to these authors protein quality and level of intake may have a significant effect on the saccharo-proteolytic flora in the calf intestine, in particular the E. Coli population. Poor quality diets, obtained by heat treatment of the skim milk used,

enhanced the rate of build-up of E. Coli infection in susceptible calves. As a result, an increased incidence of scours and a higher mortality rate was observed. The heat treatment had denaturated the whey proteins in the milk almost completely. The authors concluded that the denaturation, associated with a reduction of ionizable calcium and release of SH-groups, and the poor clotting ability by rennet were mainly responsible for the observed disorders. Other investigators confirmed the importance of the whey protein fraction in the prevention of Enteric Colibacillosis in milk-fed calves (INGRAM et al., 1970; LOGAN et al., 1974). In further studies TAGARI et al. (1969) and TERNOUTH et al. (1974) investigated the importance of milk clotting in diet digestibility and the prevention of putrefactive diarrhoea, using heat processed skim milk powder in their experimental diets. The reduced clotting enhanced the pH in pyloric chyme and reduced the pancreatic secretion and the apparent digestibility of the diet in the small intestine. The higher nitrogen flow into the lower intestine was considered to have stimulated the growth and multiplication of E. Coli.

These results confirmed the general view in these years that the protein fraction in calf's diet was one of the most critical components in nutritional scouring. Particularly the inefficient clotting in the abomasum was considered to be an important factor in this respect. It would disturb the normal pattern of chyme flow and reduce the enzymatic activity in the intestinal lumen, which consequently would reduce diet digestibility and induce (putrefactive) diarrhoea (91, 141, 152). More recent experiments did, however, not confirm this opinion. Feeding pre-clotted and re-homogenized milk did not provoke scours in calves (26, 31, 135, 136, 181, 182, 191), neither did non-clotting proteins like soya protein, whey protein or its derivatives (60, 61, 62, 121, 168, 169).

Fats

The literature on the relationship between dietary fat and the occurrence of scouring is by no means conclusive. Particularly in earlier publications a higher incidence of scours was usually considered to occur when the fat intake increased, or the nature of the fats changed (2, 3, 15, 43, 63, 87, 88, 91, 187). The publications give, however, evidence to presume that in these experiments rancidity, insufficient emulsification, and/or specific toxic constituents have been the main reasons for the poor performances observed.

Other trials showed that the addition of fat to milk replacer diets had a favourable influence on the occurrence of scouring (26, 76, 104, 132, 135). The high incidence of scouring on the control, low-fat diet was, however, presumably primarily associated with high carbohydrate intake. The favourable effect of dietary fat, replacing these carbohydrates, seems therefore not unusual.

Carbohydrates

Fermentative diarrhoea due to high carbohydrate intake seems quite common in milk-fed calves, at least if the number of publications dealing with this relationship is assumed to be representative of its incidence. A more detailed review of the literature is given in Chpt. 4.

Level of feed intake

Usually the level of intake of a milk substitute diet, or the milk concentration is believed to influence the incidence of scours. PETTYJOHN et al. (1963) investigated the effect of milk concentration on scouring in calves aged two to four weeks. The concentrations varied from 5 to 25%. They observed more diarrhoea and a reduced dry matter (DM) and nitrogen digestibility when the concentration was 15% or higher and the daily DM intake increased from 2 to 3% of BW. The rather low digestibilities observed in this experiment, however, indicate that the results may not be completely representative for modern milk substitute diets.

WISE et al. (1968) reported no effect on scours in young calves when the daily liquid milk intake increased from 7–10% of BW. Ad lib intake (13.7–18.5% of BW), however, increased the incidence of scours considerably, which could partly be prevented by increasing the frequency of feeding. More recently, BURT et al. (1972) observed a lower faecal DM content, when increasing the milk concentration from 10 to 25%, or diluting the diet to 5.5% DM. However, clinical diarrhoea was not observed, either by BURT et al., or by MYLREA (1966[a, b, c]) in experiments with fistulated milk-fed calves. The latter author noticed a remarkable ability of the new-born calf to adapt to abrupt changes in feed intake. Although the total weight of duodenal wet digesta increased with the higher level of intake (from 10 to 18% of BW per day), neither digesta flow rate (in proportion to oral intake), nor animal's health was measurably affected.

Differences in diet composition or the quality of its components may, to some extent, be responsible for the discrepances observed in the literature. But the breed of the calf used and differences in individual sensivity to dietary disturbances may also be important. Among other authors ROY (1969) indicated that e.g. Ayrshire calves tend to be more susceptible to nutritional diarrhoea than are Friesian calves.

2.5. CONCLUSIONS

The literature shows that composition and daily intake of milk can be important factors in the incidence of scouring. In particular the minerals and carbohydrates seem to be rather critical components in young, milk-fed calves. Whether or not dietary fats exert diarrhoeic properties is still questionable. Neither the relationship between 'overfeeding' and the incidence of scours is fully elucidated. The ability of calves to adapt to abruptly increased milk intakes proved to be remarkable in some experiments. Although several authors suggested that putrefactive diarrhoea is related to the diet and is frequently met in calves, it is still questionable whether in modern milk replacers nutritional factors and especially the protein fraction is of any importance in this respect.

From the literature it would appear that virtually no work has been carried out in which the diarrhoeic properties of dietary components, particularly the organic ones, have been compared. Although most authors postulate a relationship between the calf's diet and scouring, their results give very little information

about differences between diet components in their diarrhoeic properties or the maximum level of intake that can be tolerated.

3. DIET COMPOSITION AND FEEDING LEVEL IN RELATION TO DIARRHOEA. EXPERIMENTAL RESULTS

3.1. INTRODUCTION

One important question, to which the literature provided no satisfactory answer, was that concerning the different diarrhoeic properties of individual organic components of the diet. This is very relevant in determining the choice of ingredients suitable for inclusion in milk replacers. Consequently we carried out a series of experiments to get more information on this point.

In the first trial (Exp. 1) the diarrhoeic properties of milk substitute diets, varying in composition or level of intake, were investigated. In Exp. 2 special attention was paid to the relationship between clotting of the milk in the abomasum and faecal consistency, as the results cited in the literature in this respect were rather conflicting. In three successive experiments (Exp. 3, 4 and 5) the effect of high concentrations of dietary components in the lower intestine on the incidence of scours was investigated.

All experiments were carried out at the Institute for Animal Nutritional Research (ILOB) at Wageningen.

3.2. GENERAL EXPERIMENTAL PROCEDURES

Our approach differed from that usually in standard feeding studies, in that we considered any attack of diarrhoea as a departure from normality, regardless of the statistical significance of such an occurrence. Therefore it was not our primary objective to measure characteristics of animals or faeces in calves suffering from diarrhoea for a prolonged time. We were more interested in the digestive changes occurring, when the calves were abruptly changed over to a milk replacer diet which had proven to induce scours. When the young animals were changed to such diets, scouring usually started within one or two days. The experimental diets were therefore fed for only short 'experimental periods'*) usually 3–5 days, so that the general health of the calves was affected as little as possible. Sampling started immediately after the first feeding in each P. Between two successive periods a control diet was fed allowing the animals to recover from the intestinal disturbances induced. In almost all experiments calves recovered within one or two days from the nutritionally induced diarrhoea.

Calves, feeding regime and housing

Only bull calves were used in the trials, almost all of the M.R.IJ. breed,

*) Each 'experimental period' is furtheron referred to with the letter P; e.g. 'experimental period 1' is P_1, etc.

although some F.H. bull calves were used. The animals arrived at the experimental unit when they were approximately five days old and presumably had received colostrum in their early days of life.

For the first three days after arrival they received a maximum of 2 kg liquid milk replacer (260 g DM) per day, supplemented with a therapeutic dose of 20 mg penicillin-streptomycin and 20 mg furazolidone per kg BW. At the first day a massive oral dose of vitamin AD_3 was given in addition. From then on a commercial milk replacer diet was fed, gradually increasing from 260 g to 900 g dry milk substitute per day at about 3–$3\frac{1}{2}$ week after arrival.

Afterwards the animals were fed according to a standard feeding schedule generally applied at the ILOB and based upon the regression:

$$M = \frac{100\,BW^{3/4}}{ME} + 0.011\,BW$$

M = daily amount of dry milk replacer in kg; BW = body weight in kg; ME = kcal metabolizable energy per kg dry milk replacer; in our experiments usually 4400 kcal.

The dilution rate of the milk replacer depended on age and BW, but was usually 1 : 5.5–5.6 in our experiments. The animals were always fed according to this standard feeding schedule before the experiments started. The schedule was also used in some experiments, but in most experiments another feeding regime was preferred as is indicated in the Appendix.

In almost all experiments calves were kept in metabolism cages to facilitate faecal and urine collection and the handling of the fistulated animals.

Fistulation techniques

In most experiments fistulated animals were used; the re-entrant cannulae being inserted in the duodenum, the ileum or at both sites. The proximal duodenal cannula was inserted 3–5 cm distal to the pylorus and the distal cannula at about 5–10 cm distal to the entrance of the bile duct. The ileal cannulae were both inserted at the distal end of the ileum, just proximal to the ileo-caecal orifice. The techniques used are described by VAN HELLEMOND (1977).

Sampling of digesta

Preliminary experiments with duodenal fistulated calves showed that frequent sampling and returning was necessary during the collection of pyloric digesta to avoid affecting the rate of abomasal outflow. This was one of the reasons that a semi-automatic apparatus was developed for the collection, sampling and returning of the duodenal digesta (figure 1 and 2). The samples obtained were kept frozen for subsequent analysis.

Ileal digesta were collected in bottles, placed in ice. The digesta were weighed each hour and kept frozen until analysed. Unless osmolality was measured, 1 ml 40% formalin was added per 1000 g digesta. To compensate the calf for the loss

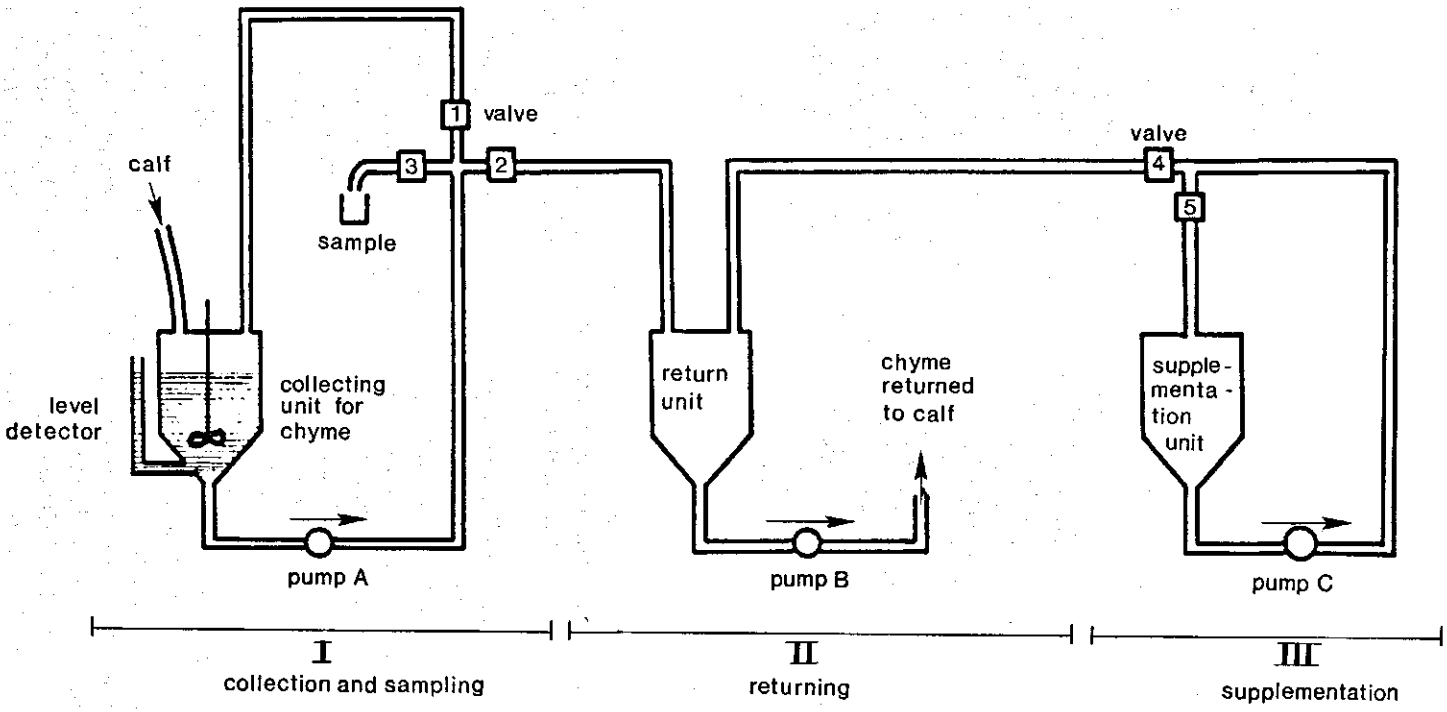

FIG. 1. Apparatus for collection and sampling chyme from calf duodenum. The chyme from the proximal cannula flows into the collecting unit, where it is micronized by a stainless sieve. Pump A moves the chyme, allowing re-circulation (valve 1) or returning and sampling (valve 2 and 3, respectively). Via valve 2 chyme is pumped into the return unit. Pump B moves chyme gradually into the distal cannula. The system of opening and closing of valves is initiated by fluid level in the collecting unit (usually fixed at 300 or 500 g chyme) and programmed for aliquot samples of 5% or any higher aliquot wanted. Samples are compensated for by a 0.9% NaCl solution moving from the supplementation unit into the return unit by pump C. During collecting, sampling and returning procedure chyme is kept at body temperature.

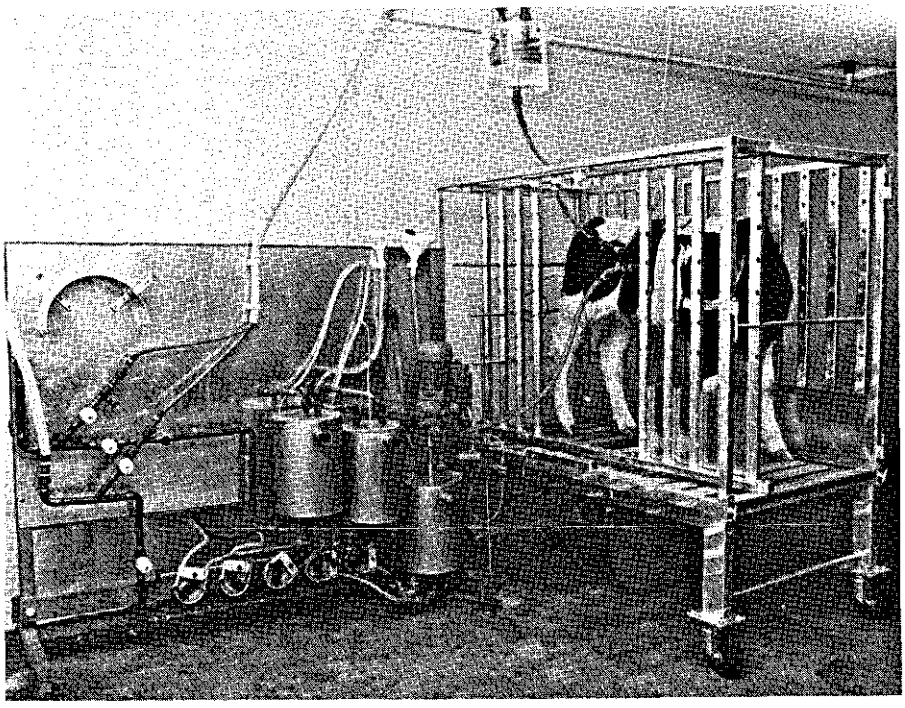

FIG. 2. Sampling duodenal digesta in calves.

of ileal digesta, an equivalent weight of a mixture of faeces, diluted with 0.9% NaCl solution to a DM content of approximately 8%, was returned into the distal cannula. The faeces used for this purpose were obtained from healthy, milk-fed calves.

The procedure described as well as the surgical technique were investigated in a series of preliminary trials, to see if they had any effect on the digestive processes. The accuracy of the duodenal sampling apparatus proved to be highly satisfactory. During 12 h of operation the mean differences between the total amount of digesta, calculated from the 5% samples, and those actually collected were 0.15 ± 1.53, 0.17 ± 1.43 and 0.23 ± 2.76% for wet digesta, DM and N, respectively, relative to the collected quantities.

The effect of the duodenal sampling technique on flow pattern and transit time of the digesta in the abomasum and small intestine was examined in another preliminary experiment using 4 calves, fitted with re-entrant cannulae in the duodenum and ileum. Digesta flow rate and transit time were measured at the ileal site when duodenal cannulae were connected to, or disconnected from the apparatus. To measure the transit time Cr-EDTA was added either to the milk or to the digesta returned into the duodenum. The pattern of ileal digesta flow (weight of wet digesta, DM and Cr) was not seriously affected by the sampling technique used for duodenal digesta. Cr recovery at the ileal site indicated that

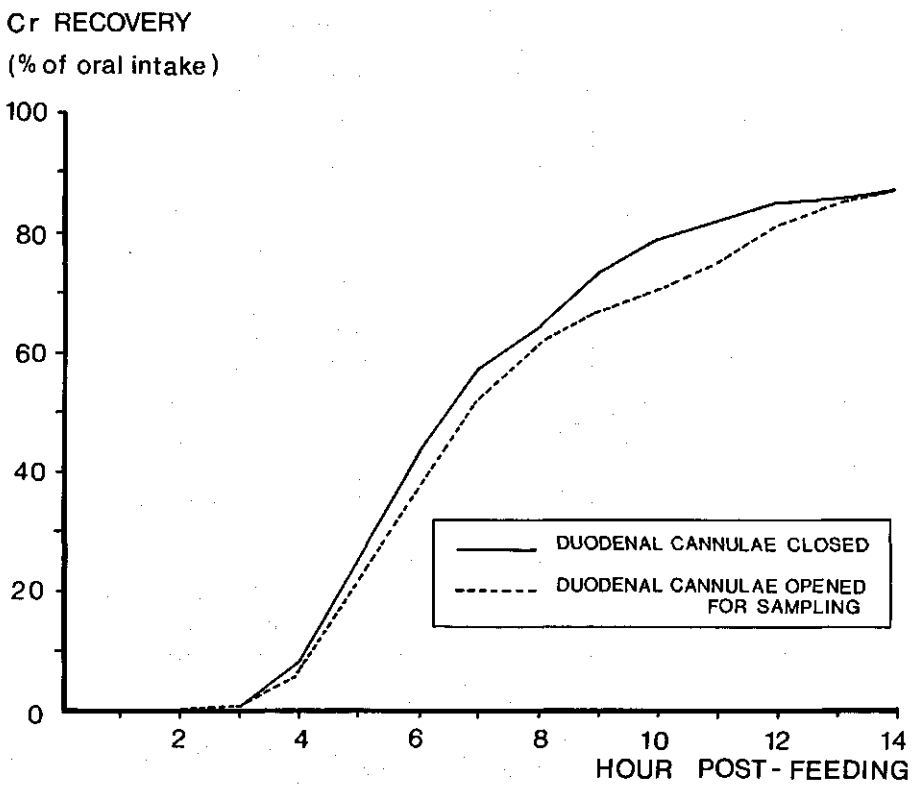

FIG. 3. Recovery of Cr from oral Cr-EDTA at the distal end of the ileum in milk-fed calves.

the duodenal sampling procedure delayed Cr arrival at the end of the small intestine by about 35 minutes (figure 3). This time lag was almost equal to the mean time span the digesta stayed in the apparatus. As the delay was almost constant and explainable, the sampling technique used seemed acceptable for our purpose. These results were in close agreement with those obtained by VAN WEERDEN et al. (pers. comm.) in a similar experiment, although they used the blood sugar curve in jugular blood as criterion for the digesta flow rate and transit time.

The effect of the surgery techniques on the digestive ability of the calf was tested in three experiments. The digestibility of the main dietary components was measured before and after the insertion of the cannulae in the duodenum, the ileum, or at both sites, using a commerçial milk replacer diet (table 1). The differences observed were only small and statistically non-significant (t-test). It was thus assumed that the surgery techniques had no measurable effects on the overall digestive ability of the calves.

Faecal collection and parameters used to measure the severity of scours

Faeces were collected quantitatively at least every 12 h. For this purpose the

TABLE 1. The apparent digestibility of a commercial milk replacer diet in non-fistulated and fistulated, milk-fed calves.

	Number of observations	Apparent digestibility (%)				
		Dry matter	Organic matter	Crude protein	Crude fat	Nitrogen free extracts
Non-fistulated calves	9	96.9 ± 0.8[1]	97.2 ± 0.6	95.0 ± 1.2	95.3 ± 2.2	99.0 ± 0.3
Fistulated calves[2]:						
duodenal cannulae	15	96.9 ± 0.6	97.4 ± 0.5	95.8 ± 0.8	95.0 ± 1.5	99.1 ± 0.2
ileal cannulae	15	97.3 ± 0.8	97.6 ± 0.8	95.1 ± 1.2	96.7 ± 2.3	99.2 ± 0.3
duodenal + ileal cannulae	8	97.1 ± 0.2	97.6 ± 0.2	96.1 ± 0.4	95.6 ± 0.4	99.2 ± 0.1

[1] Mean ± sd.
[2] Re-entrant cannulae.

animals were harnassed with plastic bags attached to the harnasses for faecal collection. All samples were preserved with a daily addition of 2 to 3 ml 40% formalin and kept frozen until analysis. When osmolality was measured, the samples were taken as frequently as possible and kept frozen without any addition.

The diarrhoeic characteristics of the faeces were measured by subjective and objective parameters. At the moment of faecal sampling their consistency was judged visually and classified in three groups:

'N(ormal)': no apparent loose faeces,
'L(oose)': watery faeces still possessing a certain substantiality,
'D(iarrhoea)': watery faeces without any substantiality.

In addition to the visual score, faecal pH (measured at the same moment) and, frequently, faecal DM content were used as quantitative parameters for the severity of scours.

Rectal temperature was checked regularly to differentiate between nutritional diarrhoea and other clinical disorders caused by infective agents.

Chemical methods

Diets, faeces and digesta were analysed for dry matter (DM), ash, nitrogen and crude fat (EE) according to standard methods. The samples were dried in an oven at 101° C to constant weight for dry matter analysis; ash was determined by incineration at 550°C during 4 h and total N content was analysed in fresh material, using a Technicon Auto-analyser. After wet digestion with 2.0 M potassium sulphate solution in 18 M sulpheric acid and selenium as a catalist, the nitrogen was bound by hypochlorite and phenol, according Berthelot. The nitrogen-complex was measured at 630 Nm. Crude fat was analysed by treating for 1 h with 3 M hydrochloric acid and drying for 3 h under vacuum at 100°C, followed by 8 h extraction with diethyl-ether. Nitrogen free extract (NFE) was calculated as DM – (cr. ash + N × 6.25 + EE), except in dietary samples, where the crude protein (CP) was calculated as N × 6.38.

In some trials electrolytes were analysed after incineration; the ash was solved in ca. 4 M hydrochloric acid and diluted with demineralized water to standard concentrations. Ca and P were analysed on the Auto-analyzer; Ca at 570 Nm after addition of dimethyl cresolphtalein-complex and P at 660 Nm after adding ascorbic acid and ammonium molybdate. Mg, Na and K were analysed by atomic absorption spectrophotometry; Fe was determined with a spectrophotometer at 516 Nm after additions of Na-acetate, hydroxyl-amine-hydrochloride and 1,10 – phenantroline.

Osmolality in digesta and faeces was measured with an osmometer in the filtrate obtained after ultrafiltration through cellophane (VAN WEERDEN, 1959). Preliminary tests showed reproducible osmolalities when the samples were kept at –20° C and the ultrafiltrates at 0°C.

3.3. The effect of diet composition and level of feed intake on faecal characteristics

In Exp. 1 the effect of diet composition or its level of intake on faecal consistency, pH and DM content was investigated in twelve subsequent periods, each of three days ($P_1 - P_{12}$; see Appendix, page 114).

Six animals received a commercial milk replacer intending a daily intake of 17 g dry milk replacer per kg BW, that supplied 8 g Hex. Eq.*) per kg BW. Lactose was added to this diet in five experimental periods. The extra amount Hex. Eq. varied from 37.5–125% of that ingested with the control diet. The lactose treatment was repeated to enable the measuring of an effect of age on faecal response to high milk sugar intakes. In another experimental period sucrose was added at levels of 37.5 and 75% above the Hex. Eq. intake with the control diet. Both levels resulted in severe scouring. To get more information on the limit of sucrose that is tolerated, the treatments were stopped after two days. After one day for recovery the initial sucrose levels were changed into 18.75 and 37.5% sucrose, which were given for another two days. Gelatinized and raw corn starch were additionally supplied in P_7 and P_8 at levels ranging from 12.5 to 75% of the Hex. Eq. intake with the control diet. In P_{10} and P_{11} milks, containing three levels of protein or fat, respectively, were fed to test their diarrhoeic effects. The high intake of the control diet itself, 'overfeeding', was tested in P_3, increasing the daily allowance by 37.5 or 75%. More detailed information on the experimental design and composition of the diets is given in the Appendix, page 114.

When nutritional diarrhoea was induced, it usually occurred within one or two days after starting the treatments. The scouring did not result in notable disorders in the general health of the animals. They recovered quickly when the treatments stopped.

The addition of lactose or sucrose to the control diet decreased faecal consistency in most calves (table 2). Faecal pH and DM content were also lower, especially when the highest levels were fed. Lactose addition of 75% (6–7 g Hex. Eq. per BW extra) reduced the frequency of *Normal* scored faeces by about 30%; higher supplementations (10 g Hex. Eq. per kg BW extra) resulted in as much as 50% incidence of scours. Sucrose readily induced scours. Almost all faecal samples collected at the highest levels of this treatment were classified as *Diarrhoea*. The quantitative faecal parameters, pH and DM content, closely reflected the changes in consistency, both decreasing when faeces became more watery (figure 4). In both sugars a significant relationship existed between the pH and DM content ($r = +0.80$ and $+0.84$ for lactose and sucrose, respectively). In the sucrose treatment the scouring started earlier and was much more serious than in the high lactose treatments. Responses in faecal characteristics were observed within 12 h after the first sucrose intake, while measurable effects of lactose usually started after two or three feeding times. Statistical analysis of the pH and

*) According to WALKER (1964), the carbohydrate content of the diet was expressed in terms of Hexose Equivalent (Hex. Eq.); e.g. lactose values were converted into the equivalent weight of monosaccharide and expressed as hexose sugar.

TABLE 2. The average daily intake of carbohydrates and its effect on faecal characteristics (Exp. 1).

Treatment		Actual daily intake (g Hex. Eq./kg BW)			Faecal characteristics				
Carbohydrate added to the control diet	Intended level of addition[1]	Control diet	Addition	Total	Visual score[2]			Faecal pH	Faecal DM (%)
					N	*L*	*D*		
Lactose	0	7.6 ±0.3[3]	–	7.6 ±0.3	21	3[4]	–	7.7 ±0.1[a]	13.62 ±1.69[a]
	37.5	7.4 ±1.0	2.8 ±0.1	10.2 ±0.9	35	1	–	7.1 ±0.6[b]	10.27 ±2.12[b]
	75–87.5	7.3 ±0.9	6.3 ±0.4	13.6 ±0.8	28	8	9	5.7 ±1.0[c]	8.71 ±1.64[c]
	125	7.4 ±1.0	9.4 ±0.2	16.8 ±1.0	15	3	17	5.5 ±1.4[c]	8.84 ±3.06[c]
Sucrose	0	8.0 ±0.1	–	8.0 ±0.1	5	–	–	8.0 ±0.4[a]	11.64 ±1.13[a]
	18.75	8.0 ±0.2	1.4 ±0.0	9.4 ±0.2	2	1	–	6.2 ±1.4[b]	9.14 ±2.21[ab]
	37.5	7.8 ±0.2	2.8 ±0.1	10.6 ±0.2	1	1	5	5.3 ±0.6[b]	7.71 ±1.72[b]
	75	8.1 ±0.1	5.8 ±0.1	13.8 ±0.2	–	–	5	5.4 ±0.4[b]	7.71 ±0.29[b]
Gelatinized corn starch	0	8.0 ±0.0	–	8.0 ±0.0	4	–	–	7.3 ±0.3[a]	9.10 ±0.62[a]
	37.5	7.7 ±0.4	2.8 ±0.1	10.8 ±0.1	5	–	2	5.1 ±1.6[b]	10.27 ±1.28[ab]
	75	8.0 ±0.1	5.6 ±0.0	13.6 ±0.1	4	3	–	5.4 ±1.9[ab]	12.29 ±2.27[b]
Raw corn starch	0	7.9 ±0.2	–	7.9 ±0.0	10	–	–	7.4 ±0.2[a]	10.00 ±1.66[a]
	12.5	7.9 ±0.0	0.9 ±0.0	8.8 ±0.0	4	–	–	7.5 ±0.1[a]	10.90 ±1.48[ab]
	30	8.0 ±0.1	2.2 ±0.0	10.2 ±0.2	5	3	3	5.8 ±1.2[b]	12.88 ±1.53[b]
	75	7.7 ±0.0	5.2 ±0.1	13.0 ±0.1	2	1	4	5.5 ±1.7[b]	15.46 ±4.33[b]

[1] Additional quantity of Hex. Eq. as % of the amount present in the control diet (see Appendix, page 114).
[2] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.
[3] Mean ± sd. Means not sharing a common letter differ significantly within the carbohydrate tested.
[4] One calf, suffering a mild lung infection in P_1, excreted three *Loose* samples.

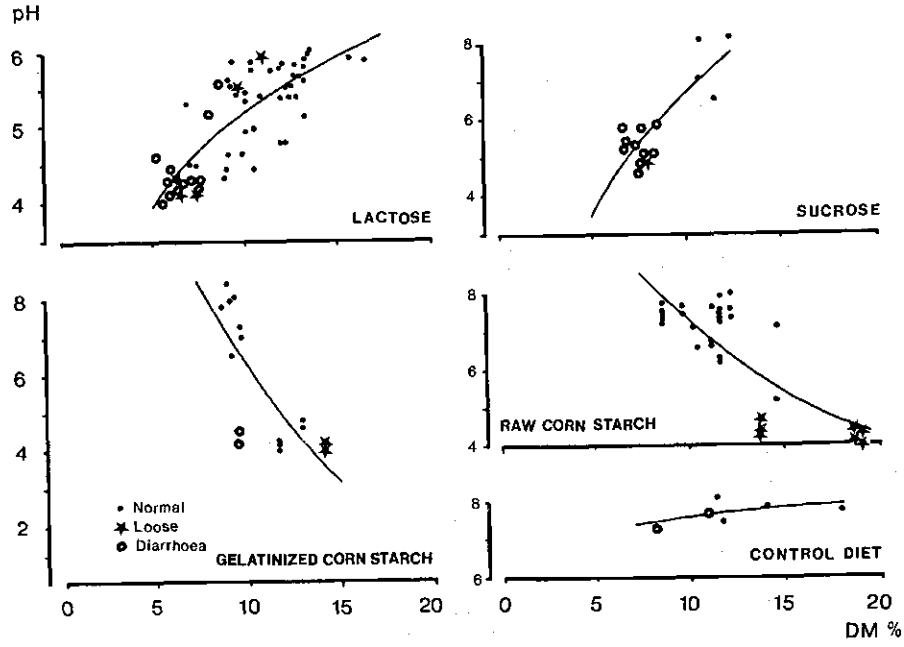

FIG. 4. The relationship between faecal pH and DM content, varying the daily intake of carbohydrates or liquid milk (Exp. 1).

DM data did not indicate any effect of age on animal response to high lactose intakes in this experiment, as was supposed by e.g. HUBER (1969). Changes in lactose tolerance seemed to be more related to diet adaptation than to age, as was also reported by GROPP (1973) and COOMBE et al. (1974).

The starches apparently affected faecal characteristics in a similar way, but significant changes did not occur before the third feeding time. The level of 1 g Hex. Eq. raw starch per kg BW in addition to the control diet did not affect faecal characteristics during two days treating. After one day recovery this amount was increased to 6 g Hex. Eq. per kg BW (75%) for another two days. That level induced scours and a fall in faecal pH. Surprisingly, faecal DM content increased with the higher levels of starch intake. The correlation between faecal pH and DM content was negative ($r = -0.78$) in the starch treatments, contrary to the results obtained with the sugars lactose and sucrose. This phenomenon may to some degree have been caused by the relatively low DM content in the faeces of the animals receiving the control diet (table 2). But a positive effect of starch on faecal DM content can not be excluded. BOEKHOLT (1976) observed a similar increase when infusing starch in ileo-caecal cannulae in adult cows. In his experiments faecal analysis indicated that the higher DM content was partly the result of an enhanced faecal N excretion, but probably also of a higher excretion of mucous substances. In fact the faeces sampled in our experiments had a rather mucous appearance.

TABLE 3. The average daily intake of crude protein or crude fat and its effect on faecal characteristics (Exp. 1).

Treatment		Actual daily intake (g/kg BW)	Faecal characteristics				
Added component	Intended level of addition[1]		Visual score[2]			Faecal pH	Faecal DM (%)
			N	*L*	*D*		
Casein	0	4.3 ±0.0[3]	6	–	–	7.6 ±0.3[a]	13.82 ±4.34
	70	7.3 ±0.1	8	–	–	7.4 ±0.3[ab]	13.42 ±3.87
	140	10.6 ±0.2	4	–	–	7.2 ±0.1[b]	15.34 ±5.83
Fat	0	3.4 ±0.0	10	–	–	7.6 ±0.4[a]	14.98 ±4.75
	35	4.4 ±0.4	6	1	1	7.3 ±0.6[ab]	14.56 ±4.38
	60	5.4 ±0.2	5	3	–	7.1 ±0.5[b]	13.47 ±3.85

[1] Additional quantities of CP and EE as % of the respective amounts in the control diet (see Appendix, page 114).
[2] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.
[3] Mean ± sd. Means not sharing a common letter differ significantly within the components.

As far as the visual score and pH of the faeces was concerned the response to starch intake was delayed by about 24 h compared with lactose. It might indicate that the microbial flora needed more time to adapt to starch than to milk sugar in this experiment. The design was not such as to enable us to determine whether or not a longer adaptation period than two to four days would have increased the fermentation rate of the starches and had changed the DM content in the faeces.

Increasing the casein content with 140 % in addition to that in the control diet only slightly affected faecal characteristics (table 3). The small, but significant shift in pH from 7.6 to 7.2 seemed to indicate a higher rate of saccharo-proteolytic fermentation. The increased fermentation, however, seemed not to interfere with faecal consistency. Neither did the results suggest an increased risk of putrefactive diarrhoea, which, according to the literature, is characterised by the shift to a higher faecal pH.

The high fat intakes resulted in four samples of *Loose* faeces and one *Diarrhoea* sample. It indicated that this diet component had more diarrhoeic potential than the protein fraction. The changes in pH and DM content were not consistent; faecal pH was only at the highest level of fat intake slightly decreased. As with the starches, the faecal response was retarded in relation to the sugars and did not start before the third day of the experimental period. It was therefore decided to extend the fat treatments for another day. The figures in table 3, showing the effect of these treatments, thus represent the average results of four days of treating instead of three. It seemed likely that the microbial flora in the intestine only adapted gradually to the high fat intakes. This was supported by the statistical analysis of faecal DM data, showing significant differences between the days of treatment. Mean faecal DM content decreased during the experimental period from 15.42 to 12.79 % when 35 % more fat was supplied and

TABLE 4. The average daily intake of the control diet and its effect on faecal characteristics (Exp. 1).

Treatment[1]	Actual daily intake (g milk substitute/kg BW)	Faecal characteristics				
		Visual score[2]			Faecal pH	Faecal DM (%)
		N	*L*	*D*		
0	16.8 ±0.2[3]	6	–	–	7.9 ±0.2[a]	14.61 ±3.60[a]
37.5	22.7 ±0.1	3	3	–	7.7 ±0.2[ab]	12.42 ±1.67[ab]
75	28.0 ±0.6	3	3	–	7.4 ±0.2[b]	9.83 ±1.94[b]

[1] Intended level of additional intake as % of control diet.
[2] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.
[3] Mean ± sd; means not sharing a common letter differ significantly.

from 16.19 to 12.56% on the highest fat treatment.

The results obtained in P_3, when high amounts of control diet were offered, are summarized in table 4. The actual milk intakes in this period were 0, 34 and 67%, respectively, above that of the control diet and were thus slightly lower than was intended. The high levels of intake resulted both in 50% *Loose* faeces, indicating that the animals suffered 'overfeeding'. The changes in faecal DM content closely followed the results of the visual score. Faecal water content increased with decreasing consistency. The faecal pH, however, was not substantially affected and remained rather high and in the range of *Normal* faeces.

These differences in the response of faecal pH and DM content made it difficult to determine the diet component primarily responsible for the *Loose* faeces observed in this experimental period. At comparable levels of intake (8.1 and 13.5 g Hex. Eq. per kg BW), lactose would have reduced faecal pH from 7.7 to 5.5 and faecal DM content from 13.6 to 8.7%. Equal fat intakes (3.2 and 5.3 g per kg BW) would have resulted in changes from 7.6 to 7.1 and 15.0 to 13.5%, respectively. In relation to the results in high milk intakes (table 4) these figures indicate that an excess of lactose reduced faecal DM content in a comparable extent. However, the changes in pH reflected almost those observed in the high fat treatment.

This discrepancy strongly suggests an interference between the diet components in their diarrhoeic effects. The data collected in Exp. 1 did not give any information whether the interaction took place in the small intestine (interference in digestion and absorption) and/or in the colon (interference in microbial fermentation), although the latter one seems to be the more probable.

The response of the calves to the separate components (i.e. carbohydrates, casein and fat) added to the control diet indicated that faecal consistency and pH fell rapidly when the carbohydrate intake increased. Sucrose and starches proved to be rather critical in this respect, although the response to starch was slightly delayed. The increase of lactose in the milk diet induced also readily thin faeces. High concentrations of well homogenized fats of high nutritional quality may

become critical too. Although in this experiment the effect was obviously delayed and less clearly defined than with similar relative increases of the carbohydrate intake. High casein intake did not affect faecal characteristics in this experiment.

Although the responses of the calves to the separate treatments were evident, they were less informative about the quantitative differences in diarrhoeic properties of the components. This may partly have been due to the limited number of calves used and to the short experimental periods. But the interpretation is made more difficult by the fact that the parameters used to measure faecal response quantitatively,pH and DM content, were inappropriate for comparing the diarrhoeic properties of the individual diet components.

Faecal pH was usually closely related to visual score when high levels of carbohydrate were fed. This parameter, however, was hardly informative about the effect of high fat or protein intake. According to the literature, faecal pH depends mainly on the type and extent of microbial fermentation in the large intestine. This fermentation is rather specific to the substrate and consequently to diet composition. Faecal pH may thus serve as a useful parameter for the severity of scouring for specific diet components, e.g. carbohydrates.

A diagnosis based on DM content alone may also lead to misinterpretation of the severity of scours, as was demonstrated when starch was fed. This criterion does not always reflect the seriousness of the diarrhoea. The DM content in faeces may simultaneously be affected by a higher excretion of components exerting no osmotic effects, e.g. mucous substances.

3.4. The role of abomasal clotting in diarrhoea

In Exp. 1 excessive casein intakes did not affect faecal characteristics. Unlike that in the experiments cited in the literature, where scouring was related to protein intake, the casein used in our experiment had normal clotting properties. This may have been the reason for the absence of an adverse response.

In Exp. 2 we tried to test the importance of clotting in the abomasum in the diarrhoeic effect of milk diets. For this purpose a liquid milk replacer was directly infused into the duodenum, using two calves fitted with re-entrant cannulae at that site (see Appendix, page 116). The proximal cannula was attached to the duodenal collecting apparatus and abomasal digesta was collected quantitatively. Instead of the collected chyme, an equal quantity of the liquid diet, control diet A, was returned into the distal cannula. To compensate for the saliva and abomasal secretory additions, according to previous experiments amounting to approximately 70% of oral intake, the milk infusate was diluted with a 0.4% NaCl solution to 170% of oral liquid milk intake.

During the days of treatment abomasal flow rate usually proved to be lower than was expected. It resulted in lower infusion rates of (dry) milk replacer compared with the oral intake (table 5). The decrease in abomasal emptying was presumably caused by the infusion of milk into the duodenum. Bell et al. (1978) reported similar experiences when milk components were infused into the duo-

TABLE 5. Feed intake, milk infusion into the duodenum and the effect on faecal characteristics in Exp. 2.

		Daily intake (g milk substitute/kg BW)		Faecal characteristics			
				Visual score[1]			Faecal pH
		Oral	Infused[2]	*N*	*L*	*D*	
P_1	calf 1	15.8 ±3.8[3]	12.0 ±2.6	7	–	–	7.8 ±0.3
	calf 2	15.4 ±2.8	13.0 ±1.6	7	–	–	6.9 ±0.4
P_2	calf 1	17.0 ±1.6	14.0 ±1.8	8	–	–	7.4 ±0.4
	calf 2	9.6 ±5.8	10.6 ±2.2	7	–	–	7.4 ±0.3

[1] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.
[2] The average pyloric outflow was lower than the expected 170% of oral intake. Because the dilution of the infusate was fixed upon 170% of oral liquid milk intake, the amounts of milk substitute infused were usually lower than the oral intake.
[3] Mean ± sd.

denum. It is not unlikely that the reduced abomasal emptying was responsible for the lower milk intake, which was especially noticed in P_2.

In the first experimental period (P_1) no deleterious effects were noticed in 4 × 12 h infusion (table 5). Milk refusals, however, tended to increase at the end of this period, while faeces consistency seemed to become more firm. Both tendencies were more pronounced in P_2 when the liquid milk infusion lasted 4 × 24 h. The experimental procedure obviously affected animals' health. The calves behaved rather apathetically at the end of P_2 and feed intake was substantially reduced, especially in calf 2. Faeces became extremely firm; the animals obviously had problems in defaecating. (Because the classification used did not differentiate such firm faeces, they were scored as *Normal*; table 5). Faecal pH was not affected.

These results agree with those of VAN WEERDEN et al. (1977), feeding a pre-clotted, homogenized milk diet, and prove that clotting is not essential in the prevention of scouring in calves. The results even indicate that non-clotted milk proteins may act beneficial in reducing diarrhoea.

3.5. THE EFFECT OF THE CONCENTRATION OF DIETARY COMPONENTS IN THE LOWER INTESTINE ON FAECAL CHARACTERISTICS

The relationship between the composition of colonic digesta and faecal characteristics was investigated in three experiments (Exp. 3, 4 and 5; Appendix, page 117, 119 and 120). The higher inflow of dietary components into the colon was simulated by the infusion of graded levels of these components into the distal end of the ileum. The experiments involved in total fifteen calves, fitted with re-entrant ileal cannulae. Faecal characteristics and the faecal and ileal apparent

TABLE 6. The effect of component infusion into the lower intestine on faecal characteristics (Exp. 3, 4 and 5).

Treatment[1]		Faecal characteristics					
		Faecal pH			Faecal DM (%)		
		Control diet	Control diet + ileal infusion	Mean difference[2]	Control diet	Control diet + ileal infusion	Mean difference
Casein	5%	7.6 ±0.1[3]	8.2 ± 0.2	0.6 ± 0.2***	16.55 ±2.13	13.63 ±3.26	−2.92 ±3.65
	10%	7.6 ±0.1	7.9 ±0.3	0.3 ±0.3	16.96 ±1.87	14.34 ±2.21	−2.62 ±3.67
Fat	5%	7.6 ±0.1	7.8 ±0.1	0.2 ±0.2	18.41 ±2.00	18.99 ±0.95	0.58 ±1.05
Lactose	5%	7.8 ±0.2	7.8 ±0.2	0.0 ±0.1	16.18 ±2.18	15.60 ±2.05	−0.58 ±4.24
	10%	7.6 ±0.1	7.2 ±0.4	−0.4 ±0.3	17.68 ±0.60	15.18 ±2.95	−2.50 ±2.89
	20%	7.8 ±0.4	5.2 ±0.2	−2.6 ±0.2***	16.14 ±2.13	10.00 ±2.14	−6.14 ±0.01*

[1] Components infused in ileal cannula; the figures represent the levels of infusion expressed as % of oral component intake.
[2] Mean differences between the data obtained during the periods of infusion and those when only control diet was fed.
[3] Mean ± sd. Significancy (t-test) is indicated by: * ($P \leqslant 0.05\%$) and *** ($P \leqslant 0.001\%$). Because the calves suffering leakage were omitted, the mean values were derived from 7 animals when casein, 3 animals when fat and 2 animals when lactose was infused.

digestibility of a commercial milk replacer diet, as well as their changes during the infusions were measured (see Appendix).

Three dietary components, casein, fat and lactose, were infused proportional to their respective intakes. Casein was tested at two N levels, 5 and 10 % of oral N intake, respectively; an intended infusion of 20 % N level resulted in immediate blockage in, and leakage around the cannulae. For technical reasons only 5 % fat infusion could be realized. The fat mixture was the same as that used in the milk substitute. Lactose was infused in Exp. 5 at three levels; 5, 10 and 20 % of oral NFE intake.

The health of the calves was satisfactory when the nutritional scouring effect was not taken into account. In Exp. 3 occasionally leakage around the cannulae occurred, in particular when casein was infused. This was probably caused by blockage of casein in the cannula. Changing the infusion technique in Exp. 4 largely prevented leakage. The data obtained from calves with leaking cannulae were not included in the results, which are summarised in table 6 to 8.

Table 6 shows the effect of the infusions on the faecal parameters. No abnormalities in faecal consistency were observed when the control diet was fed. Nor did faecal disturbances occur when casein or fat was infused. Faecal pH and DM content generally confirmed the visual classification in these treatments. Only the 5 % casein infusion level increased faecal pH from 7.6 to 8.2. The shift in pH seemed, however, not to be important. Neither the visual score or faecal DM content was affected by the 5 % casein treatment, nor was any effect observed when 10 % casein was infused. The highest level of lactose infusion, 20 % of oral NFE intake, reduced all faecal parameters, resulting in some samples of *Loose* faeces with the pH decreased by 2.6 and the DM content by 6.1 units.

Statistical analysis did not prove conclusively that the animals, or their microflora adapted to the treatments during the five days of treatment. Day effects

TABLE 7. The apparent ileal and faecal digestibility of the diet components (Exp. 3, 4 and 5).

Component	Ileal apparent digestibility (%)	Faecal apparent digestibility (%)	Mean difference[1] (%)
DM	94.0 ±1.0[2]	97.3 ±0.9	3.3 ±1.2***
N	92.6 ±1.1	95.1 ±1.2	2.5 ±1.4***
EE	97.9 ±1.5	96.7 ±2.3	−1.1 ±1.6*
NFE	94.3 ±1.7	99.1 ±1.3	4.8 ±1.8***
	Mineral absorption coefficient (%)		
Ca	87.5 ±4.4	87.6 ±4.0	0.1 ±4.3
P	96.3 ±1.9	96.5 ±0.9	0.2 ±2.2
Na	66.0 ±9.5	99.6 ±0.2	33.5 ±9.4***
K	96.3 ±1.2	99.1 ±0.4	2.8 ±1.1

[1] Mean difference between faecal and ileal digestibility. The mean values of the organic and anorganic components were calculated from 15 and 6 calves, respectively.
[2] Mean ± sd. Significancy (t-test) is indicated by: * ($P \leq 0.05\%$) and *** ($P \leq 0.001\%$).

usually proved to be non-significant (F-test). Only the 10% lactose infusion resulted in a significantly lower faecal pH on the fifth day of treatment.

The milk substitute diets fed in these experiments proved to be highly digestible (table 7). The digestion and absorption of most dietary components were almost completed in the small intestine. The apparent ileal digestibility of DM averaged approximately 95%; only about 3.5% disappeared in the large intestine. This latter amount consisted mainly of NFE and, to a much smaller extent, of N and electrolytes. These results agree closely with those cited in the literature. TAGARI et al. (1969) found 91–92% of the ingested N and MORILL et al. (1965) 94% of the lactose apparently digested in the small intestine. VAN WEERDEN et al. (1977) observed that approximately 4% of the dietary N and 5% of the NFE disappeared in the colon. They found, in agreement with our results, a higher fat excretion in the faeces than the amount flowing into the hind gut; the difference was ca. 1% of oral fat intake. The higher faecal fat excretion was approximately 39 mg per kg BW per day in our experiments. That amount was about equal to that of metabolic fat excretion, estimated by VEEN (1970) as 18–64 mg fat per kg BW. If so, metabolic fat may originate mainly in the large intestine.

The mineral absorption in the control diet was rather high in our experiments in relation to that in adult animals (table 7). These results, however, are not unusual in young calves. The absorption of Ca, P and K was almost completed in the small intestine, but ca. 33% of the ingested Na was absorbed in the hind gut. The results reflect the figures quoted by other scientists. SMITH (1962) and MYLREA (1966[b]) reported also a high absorption of Na in the lower intestine of milk-fed calves. The latter author observed that 53 and 95% of Na and K, respectively, were absorbed in the small intestine. VAN WEERDEN (1959), VAN 'T KLOOSTER (1967) and ROGERS et al. (1969) reported a high Na absorption in the lower intestine of adult cattle.

The infusions of 5 and 10% casein into the lower intestine increased the 'apparent digestibility' of DM, OM and N in the hind gut by 9, 11 and 28 units, respectively (table 8). The results indicated that all casein-N infused disappeared at the lowest level of treatment and about 90% at the highest level, when assumed the apparent digestibility of the dietary N not to be affected by the infusions. Neither faecal excretion of fat nor that of NFE was affected by the casein treatment.

The introduction of 5% fat into the lower intestine increased the crude fat 'apparent digestibility' in the hind gut by 41 units. Based on similar assumptions as with casein, it was concluded that 47% of the fat infusate disappeared in this section of the intestine. Neither N nor NFE excretion was affected measurably in this treatment.

When the lactose infusion exceeded 5% of oral NFE intake, the NFE and N excretion in the faeces were both increased. If the dietary NFE digestibility was not affected, these results indicate that only 0, 9 and 19% of the infused lactose was excreted with the faeces in the three lactose infusates, 5, 10 and 20%, respectively. The two highest levels of lactose infusion into the lower intestine

TABLE 8. The effects of component infusions into the large intestine on the 'apparent digestibility' in the lower intestine (Exp. 3, 4 and 5).

Treatment	Component	App. colonic 'digestibility' (%) at infusion levels of:[1]								
		5%			10%			20%		
			Infused			Infused			Infused	
		Control	Total	Infusate[2]	Control	Total	Infusate	Control	Total	Infusate
Casein	DM	59 ± 10[3]	68 ± 10	102 ±36	57 ± 10	66 ± 7	86 ±14			
	OM	58 ± 11	69 ± 10	107 ±32	56 ± 11	68 ± 7	95 ±12			
	N	38 ± 13	66 ± 14	104 ±30	38 ± 14	66 ± 7	89 ±12			
	EE	−51 ±107	−42 ±117		−48 ±108	−42 ±64				
	NFE	86 ± 9	85 ± 7		82 ± 8	86 ± 5				
Fats	DM	48 ± 3	55 ± 4	90 ±11						
	OM	47 ± 5	58 ± 4	107 ± 2						
	N	37 ± 19	51 ± 2							
	EE	−13 ± 5	28 ± 8	47 ± 4						
	NFE	76 ± 8	85 ± 5							
Lactose	DM	48 ± 6	75 ± 2	147 ± 8	57 ± 18	72 ± 1	96 ±33	61 ±12	64 ± 4	67 ±1
	OM	51 ± 7	77 ± 3	148 ±20	59 ± 18	73 ± 1	90 ±32	64 ±11	66 ± 6	67 ±1
	N	20 ± 2	46 ± 2		36 ± 25	26 ±10		37 ±23	−14 ± 9	
	EE	−71 ± 72	22 ± 4		−59 ± 90	−3 ±22		−8 ±17	−69 ±52	
	NFE	86 ± 3	92 ± 2	104 ±16	90 ± 2	91 ± 3	91 ± 7	86 ± 6	82 ± 8	81 ±8

[1] The 'apparent digestibility' in lower intestine $= \frac{\text{inflow in lower intestine} - \text{faecal excretion}}{\text{inflow in lower intestine}} \times 100\%$.

Diet components flowing into the lower intestine were calculated from feed intake, assuming that the ileal digestibility measured in one of the preceding periods was not changed during the infusion periods.

[2] The 'apparent digestibility' of infusate in the lower intestine $=$
$\frac{\text{infused quantity} - (\text{total faecal excretion} - \text{faecal excretion of diet components})}{\text{infused quantity}} \times 100\%$.

The faecal excretion of diet components was calculated from total app. digestibility measured in P_1, which was assumed not to be affected by the infusion.

[3] Mean ± sd. The average values were derived from 7 animals when 5% casein, 6 animals when 10% casein, 3 animals when fat, and 2 animals when lactose was infused into the lower intestine.

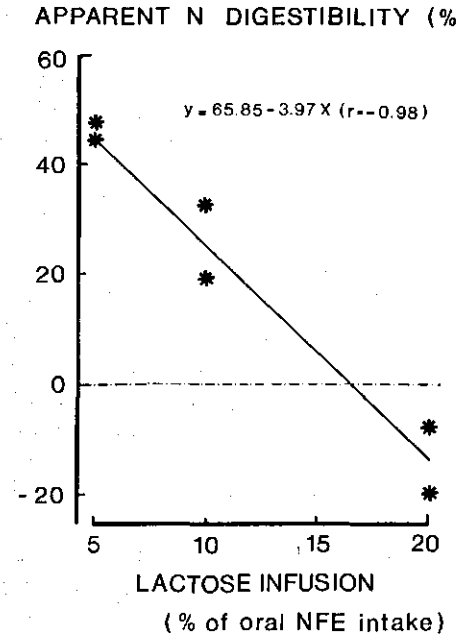

FIG. 5. The relationship between lactose infusion into the lower intestine and the apparent N digestibility in the hind gut (Exp. 5).

increased linearly the N excretion in the faeces by 15–16 mg per g lactose infused in excess of the 5% level (figure 5). It suggested a higher rate of ammonia incorporation into bacterial protein when the lactose supply increased. VAN ES et al. (1971) reported similar results. These authors suggested that the inclusion of 5% gelatinized corn starch in milk replacer diets was the maximal that could be tolerated before N digestibility was reduced.

The mineral analysis of faeces indicated that the infusates did not measurably affect the mineral absorption. This result seems to conflict with the literature, which usually reports considerable mineral losses in scouring calves. Our results may have been due to the rather mild responses of the calves to the infusions. They may, however, also be an indication that the interference of scouring in mineral absorption takes merely place in the small intestine instead of the hind gut.

3.6. GENERAL DISCUSSION AND CONCLUSIONS

The results obtained in Exp. 1–5 indicate that the increase in intake of organic compounds in milk replacers in an extent beyond those generally applied may easily result in nutritional diarrhoea in young, milk-fed calves. In particular the carbohydrates seem to be critical in this respect. Putrefactive diarrhoea induced

either by diet composition or high protein intake was not observed, at least when a shift to an alkaline pH is considered to be representative for this type of diarrhoea (WEIJERS et al., 1965). When the protein intake increased from 4.3 to 10.6 g per kg BW (146% in addition to that in control diet intake), faecal consistency nor its pH and DM content was substantially affected. Neither did an extra protein inflow into the lower intestine of 0.4 g per kg BW per day (120% in addition to normal inflow), nor the infusion of unclotted milk protein into the duodenum instead of abomasal digesta.

The apparent discrepancy between our results and those cited in the literature (e.g. ROY et al., 1959, 1964, 1969 and TAGARI et al., 1969) was presumably related to the difference in protein used. The detrimental effects in calves, cited by these authors, were observed on milk replacer diets, in which the whey protein fraction was damaged by heat treatment. That low quality protein was not investigated in our trials. These products are not important in modern milk replacer diets. Our experiments clearly show that neither the clotting of the proteins in the abomasum, nor the pH of pyloric chyme are factors in microbial activity in the hind gut resulting in putrefactive diarrhoea.

Our results with casein (and lactose) supplementation agree more closely with those of MASON et al. (1973). These authors, using diaminopimelic acid (DAPA) as a criterion, showed in laboratory animals that bacterial growth in the hind gut was not limited by the quantity of nitrogen ingested. Increasing the protein intake did not affect faecal DAPA excretion, irrespective of the N excretion. High carbohydrate intakes, however, always resulted in intensified colonic fermentation and enhanced N and DAPA excretion.

The high fat intakes resulted in less consistent responses than did casein. In Exp. 1 faecal consistency tended to become *Loose* or *Diarrhoeic* when the daily fat intake increased from 3.4 to 5.4 g per BW (60% in addition to that in the control diet). The results in Exp. 3, infusing 5% fat (0.17 g per kg BW per day, equal to 150% in addition to normal inflow) into the ileal cannula, were not conclusive as to the diarrhoeic properties of fat. However, it has to be said that the level of fat infusion investigated was lower than those of casein and lactose. On the other hand the results provide evidence that dietary fat intake may increase considerably, before a similar condition is achieved as is treated in these trials. The fat content in milk replacers is limited by technological factors, particularly in regard to its homogenizing and emulsifying properties. These quality aspects are not taken into consideration in our work. Nor is the fatty acid composition of the fats. It was assumed that these quality aspects are recognized and generally accepted as needing attention in the nutrition of milk-fed calves. Our results seem to indicate that the technological factors still impose more severe restrictions upon the quantity allowed in milk substitute diets than the diarrhoeic property of high quality fats.

High carbohydrate intake and increased carbohydrate concentration in the lower intestine evidently enhanced microbial fermention rate and reduced faecal consistency, pH and DM content. Our results confirm the general view that the diarrhoeic properties of sucrose and starch are critical in young calf nutrition.

They do not support the opinion of WALKER et al. (1964), stating that calves would tolerate a daily intake of 10 g Hex. Eq. per kg BW, irrespective of the carbohydrate used. In Exp. 1 a daily intake of 8 g Hex. Eq. lactose together with 1.4 g Hex. Eq. sucrose or 2 g Hex. Eq. starch quickly induced scouring. Neither do these results confirm the opinion of MATHIEU et al. (1965, 1968 [a,b]), that gelatinized starches are used fairly efficiently by young, milk-fed calves.

Summarizing our results, it was concluded that doubling the intake of milk protein, i.e. casein of standard quality is not detrimental to young calves. Excessive intakes of fat or carbohydrates may result in nutritional scouring. The fat concentration of the diet is, however, presumably more limited by technological capability than by the level that can be tolerated by the animals.

It was therefore decided to investigate further the effect of carbohydrates on diarrhoea and the limit that is tolerated before scouring occurs. As lactose is the most important carbohydrate in milk replacer diets, this sugar received our main attention.

4. CARBOHYDRATE DIGESTIBILITY IN MILK-FED CALVES AND ITS RELATIONSHIP WITH FERMENTATIVE DIARRHOEA. SURVEY OF THE LITERATURE

4.1. Introduction

In practice a general tendency exists to include the maximum amount of carbohydrates in milk replacer diets. The cost of protein restricts the inclusion of this fraction to the minimum needed to meet the physiological requirement for maximal growth. Besides minor additions, e.g. minerals, vitamins and antibiotics, the remaining part of the formulation is derived from fats and carbohydrates. As the fat content is limited by technological reasons, the carbohydrates are usually used to 'fill the gap' in the formulation. The level of carbohydrates is limited only by the animal's requirement for energy on the one hand and, on the other hand, by the highest level that can be tolerated consistent with normal faeces.

In Chpt. 2 is indicated that a close relationship exists between the degree of enzymatic splitting (digestion) and absorption of carbohydrates by the calf and their diarrhoeic properties. Therefore a general survey of the literature on carbohydrate digestion and absorption in milk-fed calves is given in this chapter. Finally special attention is paid to the possibility of using several different carbohydrates in milk replacer diets.

4.2. The digestion and absorption of carbohydrates

Interest in the mechanisms in carbohydrate and particularly in lactose digestion and absorption in infants and new-born animals has been renewed in the past twenty years. The discovery of hypolactasia in infants and man initiated research into the physiological and biochemical processes involved in carbohydrate digestion in humans. This work is described in several detailed reviews (9, 74, 110, 116, 140, 193, 194).

The development and improvement of milk substitute diets for new-born animals were the main influences in stimulating the re-investigation of these aspects in young animals and in particular in calves. A number of authors has reviewed this work (7, 47, 64, 85, 101, 102, 142, 169, 178). In this survey only some headlines are given.

4.2.1. *Enzymes involved in carbohydrate digestion*

Because the intestinal mucosa only absorbs monosaccharides, carbohydrate splitting enzymes are required to digest poly-, oligo- and disaccharides in the lumen of the small intestine (table 9). The starch hydrolysing enzyme α-amylase is not secreted in calf's saliva, but only by the pancreas. This enzyme acts mainly

TABLE 9. Carbohydrate splitting enzymes in the intestine (WEIJERS et al., 1965 and GROPP, 1973).

Enzyme	Substrate	End product
α-1,4-Glucan 4-glucanohydrolase (EC. 3.2.1.1.)[1]		
α-amylase	starch	maltose, isomaltose
α-D-Glucocide-glucohydrolase (EC. 3.2.1.29)		
maltase 1, maltase 2	maltose	glucose
maltase 5, isomaltase	maltose, isomaltose	glucose
β-D-Fructo-furanoside-fructohydrolase (EC. 3.2.1.26)		
sucrase 1 (maltase 3)	maltose	glucose
sucrase 2 (maltase 4)	sucrose	glucose + fructose
β-D-Galactosidase-galactohydrolase (EC. 3.2.1.23)		
lactase 1, lactase 2	lactose	glucose + galactose

[1] Enzyme nomenclature, 1972. Elsevier A'dam.

in the intestinal lumen, although some activity is observed in, or attached to the mucosa cells. GROPP (1973) and CRANE (in CZÁKY, 1975) postulate that this latter activity results from adsorption of the pancreatic amylase on the mucosal cell membranes.

Starch is split into maltose units, consisting of two glucose molecules coupled at the 1 and 4 positions, and into isomaltose, also consisting of two glucose molecules but linked at the 1 and 6 positions. Dextrins and other oligosaccharides can be hydrolysed by α-amylase, but to a much smaller extent than starch (WIDDAS, 1971).

In calves the disaccharidases are confined to the brush border cells of the intestinal mucosa and do not act in the intestinal lumen (34, 36, 37, 193, 194). In the literature three or five separate maltases are distinguished, depending on the classification of the sucrases (table 9). These enzymes hydrolyse maltose and isomaltose into their hexoses. Maltases are also able to break down other oligosaccharides and starch by splitting-off single glucose molecules from the end of the molecule. Similarly isomaltase can hydrolyse hexoses from these molecules, when coupled at the 1 and 6 positions (MC MICHAEL, 1971). In this way these enzymes may substitute α-amylase to a certain extent. Intestinal maltase activity is rather low in the calf, in particular in new-born animals. Its pattern of distribution over the small intestine is generally believed to be very irregular. HUBER et al. (1961[b]) and TOOFANIAN et al. (1973) measured the lowest activity in the duodenum.

In man and in most animals sucrose is digested by the brush border sucrase (invertase) into glucose and fructose. In calves, however, no sucrase activity is measured, even after prolonged sucrose intake (64, 77, 79, 80, 85, 102, 130, 140, 162, 179, 185).

Lactase (β-galactosidase) is the main disaccharidase in calves. KOLDOVSKÝ et

al. (1968) distinguished two separate lactases in laboratory animals. The most important one, neutral active β-galactosidase, is located in the brush border cells and exerts maximal activity at approximately neutral pH. Its activity has been extensively investigated in human research, because it is limiting in the lactose malabsorption syndrome (37, 192, 199). The second lactose splitting enzyme cited by KOLDOVSKÝ et al. is an acid active β-galactosidase with an optimal activity at pH 3 to 4. It is located in the cytoplasm of the mucosal cells and seems to be of lysosomal origin. Its nature and function in the digestion of lactose in calves is not yet understood (COOMBE et al., 1973).

TABLE 10. The average lactase activity[1] in three segments of the small intestine (HUBER et al., 1961[b]).

Age (days)	Cranial section	Middle section	Caudal section
1	1427	1828	919
8	1365	1196	173
15	1278	1447	155
22	954	913	47
33	1521	580	44
44	1242	744	52

[1] Expressed in mg glucose activity per g intestinal DM.

HUBER et al. (1961[b]) investigated in milk-fed calves the distribution of lactase activity in three gut sections of approximately equal length (table 10). Their results showed that lactase activity declined with age. The enzyme was mainly located in the proximal part of the intestine, especially when animals grew older. More recent work has confirmed these results (COOMBE et al., 1973 and TOOFANIAN et al., 1973, 1976).

4.2.2. *The absorption of monosaccharides*

Although the duodenum and to a lesser extent the ileum have the ability to absorb glucose and other monosaccharides, the jejunum proved to be the main site for absorption (33, 36, 178, 179, 193, 194). Glucose, galactose and a number of their deoxy-derivatives, when the hydroxyl group is bound at the C_2-position, are actively transferred into the intestinal wall. Current knowledge about the mechanisms involved are described in detail in CZÁKY (1975).

In-vitro experiments proved that glucose and galactose are actively absorbed. Na^+ is essential in this absorption mechanism, as it is also in the absorption of amino acids. There is much evidence available that both hexoses use the same absorption mechanism to cross the mucosal membrane. COOMBE et al. (1973) observed in calves no difference in the rate of absorption between glucose and galactose, when these were infused separately into the intestinal lumen. However, the galactose absorption was delayed when both hexoses were infused together or as lactose. It indicated a greater affinity of the transport mechanism for glucose than for galactose. That observation was confirmed by other authors (4, 32, 66, 73, 95, 162). The fact, that the galactose concentration in the lumen

does not affect glucose absorption, caused Mc MICHAEL (1971) and HONEGGER et al. (1973) to suggest that another pump, specific to glucose, was involved in active hexose absorption. CRANE (in CZÁKY, 1975) differentiated between the Na^+-dependent absorption mechanism, acting more specific for glucose absorption, and another absorption pump, also located in the digestive surface of the brush border, which acts independently of Na^+ and does not discriminate between hexoses.

In-vitro research indicated a close relationship between the enzymatic activity in the mucosal membrane and the activity of the transport mechanisms. The hexoses derived from enzymatic splitting at the brush border surface are absorbed in advance to those present in the infusate (CRANE, 1968; COOMBE et al., 1973). The former author (in CZÁKY, 1975) suggested that the enzymatically hydrolysed hexoses exert a kinetic stimulus on the Na^+ transport mechanism located in the same membrane.

On the other hand a significant, stimulating effect of higher glucose concentrations in the intestinal lumen (or infusate) on glucose absorption has been measured in several experiments (4, 8, 33, 153). CRANE (in CZÁKY, 1975) and CZÁKY et al. (1977) supposed that in particular the non-discriminating, active transport system is stimulated in that condition. However, it is not clear yet whether or not an increase of the passive hexose absorption is more likely responsible for the curvilinear relation between glucose concentration in the lumen and its rate of absorption (WIDDAS, 1971; COOMBE et al., 1973).

Much less knowledge exists on the mechanisms involved in the absorption of other monosaccharides. It is generally accepted that e.g. D-xylose, phenylglycosides, myo-inositol and L-fucose can also interact with the active absorption processes described (KIMMICH, 1973). In earlier literature the absorption of fructose and mannose is usually supposed to be a passive process. Mc MICHAEL (1967), however, did not exclude active absorption of fructose, because its absorption rate proved to be significantly faster than that of sorbose. More recently CRANE (in CZÁKY, 1975) supported this statement with the proposed active and non-discriminating 'hydrolysis-related-transportsystem', which would actively transport the hexoses without any discrimination. Mannose is still considered to be passively absorbed.

4.3. CARBOHYDRATE DIGESTIBILITY AND THE LIMIT OF TOLERATION IN MILK-FED CALVES

In general it can be stated that easily digestible carbohydrates are also easily fermented by the microbial flora in the hind gut when they reach the colonic lumen. Therefore their apparent digestibility, determined by faecal analysis, may give a misleading estimate of the quantities actually digested and absorbed by the animal itself. XENOULIS (1967), feeding C^{14}-labelled sucrose to calves, proved that the free fatty acids (FFA) from microbial fermentation were to some extent absorbed by the colon. However, the absorption was of minor importance and

contributed only slightly to the carbohydrate utilisation. Most of the fermentative end products were excreted in the faeces (XENOULIS, 1967; HUBER, 1969). On the other hand the microbial fermentation may have a considerable effect on chyme composition, increasing the amount of soluble constituents with osmotic properties. In this way it may reduce colonic water absorption and exhibit diarrhoeic properties.

For the purpose of our work the quantity of carbohydrates, actually digested and absorbed by the calf, before the interference of bacterial fermentation, was most informative. In the literature four methods are proposed for estimating this actual digestibility in the calf.

– *Growth experiments.* This method, requiring an extensive experimental period, is not an exact one. The results may be affected by dietary constituents other than carbohydrates.

– *Enzymatic activity.* Determination of the enzymatic activity in extracts of the intestinal mucosa is only representative for maximal carbohydrate absorption, when enzymatic digestion is critical in the absorption. Several authors presumed this relationship to exist in carbohydrate digestion in calves (23, 44, 45, 78, 79, 80, 81, 83, 178). However, others observed in man (MC MICHAEL et al., 1967) and in calves (COOMBE et al., 1971; GROPP, 1973[b, c]) that lactase activity was not limiting the lactose absorption in healthy individuals. Moreover, the validity of quantitative data for the digestive capacity, based on an extrapolation of enzymatic activity measured per unit weight of cell material, is extremely doubtful. For example, the effect of digesta transit time on the quantitative capacity for hydrolysis in the small intestine is difficult to estimate.

– *Carbohydrate tolerance test.* According to this test, blood sugar response is measured in the jugular vein after the intake of a standardized aqueous solution of the carbohydrate investigated (HUBER, 1961[a]). A close relationship is presumed to exist between the rate of carbohydrate absorption and the blood sugar response. The glucose concentration in the blood does, however, not only depend on its rate of absorption, but also on its clearance rate (64, 72, 128, 149, 199). Evidence exists that e.g. the hormonal regulation of the blood glucose is affected by age (9, 32, 42, 67, 89, 137, 144, 188). GROPP (1973) largely avoided this effect by sampling also the portal blood.

Another disadvantage of this method is that, according to the standard design used in the test, only carbohydrate is offered to the animal. Although BAZIN et al. (1976) did not observe any effect of dietary fat on blood sugar response, interaction between dietary components on the absorption rate of hexoses and glucose clearance cannot be neglected.

– *Apparent digestibility in the small intestine.* The object of this method is to measure directly the apparent digestibility of carbohydrates before the interference of the colonic microflora. Although some microbial fermentation may occur in the small intestine (ROY, 1969), this effect is presumed to be almost negligible. Experimental data confirmed that this method permits a much more accurate estimation of the quantity of carbohydrate actually digested and absorbed by the animal itself (35, 58, 106, 120, 124, 125, 191).

TABLE 11. The digestibility of carbohydrates in young, milk-fed calves.

Carbohydrate	Parameter used to estimate the digestibility				References
	Growth	Apparent (faecal) digestibility	Enzyme activity	Carbohydrate tolerance test	
Monosaccharides:					
glucose	variable results	complete	–	high response	23, 34, 45, 65, 78, 105, 107, 112, 162, 179, 185.
galactose	–	complete	–	comparable to glucose	34, 65, 162.
fructose	–	–	–	low response	65, 162, 185.
xylose	–	–	–	comparable to glucose	162.
Disaccharides:					
lactose	high positive response	94–98%	high	slightly lower or comparable to glucose	23, 35, 45, 58, 70, 78, 79, 80, 81, 82, 83, 114, 120, 124, 126, 127, 130, 162, 169, 178, 185.
sucrose	negative	70–90%	absent	no response	45, 65, 77, 78, 79, 119, 130, 188.
(iso)maltose	–	–	low	low response	35, 45, 66, 70, 78, 85, 130, 162, 169, 178, 185.
Polysaccharides:					
starch	negative (when at least 5% in the dry milk replacer diet)	23–98% (average ca. 70%)	low	low response	12, 23, 35, 45, 49, 54, 55, 56, 66, 70, 79, 80, 84, 85, 106, 114, 120, 121, 122, 126, 127, 129, 130, 143, 162, 169, 172, 176, 190.

The ability of new-born calves to digest and absorb the separate carbohydrates has been extensively investigated. In table 11 the most relevant results are summarised. The main monosaccharides in calf nutrition, glucose and galactose, are readily absorbed. Consequently a high tolerance for these hexoses can be expected. In tolerance tests a single dose of 4.4 g glucose or galactose per kg BW was easily tolerated, without adverse consequences on faecal consistency. Derangements were neither observed with concentrations of 5 to 7 % in liquid milk replacer diets (LISTER et al., 1973), nor from a daily intake of 9 g glucose per kg BW as a constituent of an artificial milk substitute diet (BRITT et al., 1974). Higher amounts of glucose reduced faecal DM content and readily caused scours. The disturbance was frequently associated with abnormal neuromuscular function and mortality, when the high level was fed for a prolonged period of time (55, 56, 148, 193). KLUNKER (1971) observed similar disorders in rats, together with serious hyperplasia in the duodenal mucosa after massive doses of glucose.

Scouring is also induced when high quantities of galactose (ATKINSON, 1957), or fructose (OKAMOTO, 1959) was fed to young calves. Galactose is tolerated to the same extent as glucose when its absorption is not limited by a simultaneous intake of glucose or lactose. Fructose presumably is less well tolerated because of its lower absorption rate.

Lactose is the only disaccharide well tolerated in milk-fed calves. Its apparent faecal digestibility ranges from 94 to 98 %, most of which is digested and absorbed in the small intestine (VAN WEERDEN et al., 1977). Daily intakes of 10 g Hex. Eq. per kg BW are well tolerated, but a further increase in intake, e.g. 12 g Hex. Eq. per kg BW, may induce loose faeces or diarrhoea (WALKER et al., 1964; HUBER, 1969; GROPP, 1973).

Lactose tolerance evidently depends on the extent of animal adaption to high intakes. HUBER et al. (1961[a], 1967) observed that milk-fed calves tolerated a single dose of 4.4 g lactose per kg BW in a tolerance test. The same level, however, induced serious scouring in older, ruminating animals receiving no lactose with their daily feed. Adapting these animals to a liquid diet, containing 58 % lactose and 25 % skim milk powder in the DM, rapidly increased their tolerance for lactose. SIDDONS et al. (1969) did not observe adaptive responses in lactase activity when adding more lactose to calf rations. However, BRITT et al. (1974) reported also a stimulating effect on the lactase activity in the brush border cells when the daily allowance of lactose was increased to 9 g per kg BW. According to these authors galactose would be the most effective carbohydrate in this respect in the distal section of the small intestine. They further supposed that the adaptive responses are only to expect at high lactose intakes. That could have been the reason for the lack of response reported by the former authors.

Other disaccharides are less well or even not tolerated by calves, because their digestion, and consequently their absorption, in the small intestine is rather low or negligible. Sucrose is badly tolerated at all (78, 113, 185). VELU et al. (1960) and MATHIEU et al. (1968[b]) observed severe scouring and high mortality rates after prolonged feeding of this sugar. The intensive fermentation of sucrose in

the lower end of the intestine was demonstrated in ileal fistulated calves. MORILL et al. (1963) observed 53 % sucrose apparently digested and absorbed in the small intestine. The apparent faecal digestibility was 86 %. More recently XENOULIS (1967) showed that only 1 % of the ingested sucrose disappeared in the small intestine and 87 % fermented in the hind gut.

Maltose tolerance tests in calves gave only small blood sugar responses, indicating a low absorption rate of this sugar. Frequently scours were observed when calves received a single dose of 4.4 g maltose per kg BW (78, 101, 185). The limit of calf's tolerance for maltose, however, seems to increase gradually when the animal grows older. As with sucrose, maltose intake easily stimulates microbial fermentation in the hind gut. Although several experiments indicated a promising apparent faecal digestibility of maltose, the apparent ileal digestibility ranged only from 33–70 % (HENSCHEL et al., 1963; COOMBE et al., 1974).

In practice starch, or its oligosaccharides are the most common carbohydrates, which are considered to replace lactose or skim milk powder. The low digestion of maltose in milk-fed calves, however, puts also limits on the digestion of these components. Consequently the digestion of polysaccharides does not depend on α-amylase activity alone that, in any event, is low in the new-born calf. Nevertheless, much research work has been done to evaluate the utilization of (gelatinized) starch in milk replacer diets. As is demonstrated in table 11 a large variation exists in the apparent faecal digestibility of starch, measured in calf experiments. That may be an indication for extensive fermentation of undigested starch in the lower intestine. The faecal data may therefore overestimate the actual amount digested and absorbed by the animal itself.

Growth response to starch additions seems to be variable in milk-fed calves. Most authors observed a lower growth rate when starch or its derivatives were added to diets at the expense of lactose or skim milk powder (12, 55, 56, 66, 80, 83, 122, 126, 127). Incidently, no negative effect of starch inclusion on growth rate is reported; BURGSTALLER et al. (1968) even claimed no adverse effect when 12 % starch was included in a (dry) milk replacer diet.

Adding significant quantities of starch or its derivatives to milk replacers usually resulted in scouring (77, 81, 106, 190). Similar effects were observed in sheep when starch was infused into the intestine (ØRSKOV et al., 1969; ABEL-RAHMAN et al., 1977). VAN ES et al. (1971) estimated that 5 % gelatinized corn starch might be tolerated in milk substitutes, before measurable negative effects on the apparent faecal N digestibility occurs.

It seems likely that the poor performance, generally observed when feeding significant amounts of starch, is primarily but not only due to the low activity of the starch hydrolysing enzymes. Adding α-amylase to these diets had either no effect or only slightly improved starch digestion (122, 127, 130, 143). The lack of response may, to some extent at least, be caused by the fact that the digestion of starch is not only limited by α-amylase but also by the low maltase activity in calves. VAN WEERDEN et al. (1967) investigated in calves the effect of an enzyme preparation, combining α-amylase and maltase activity, in a milk replacer containing 14 % corn starch in the DM instead of lactose. This preparation actually

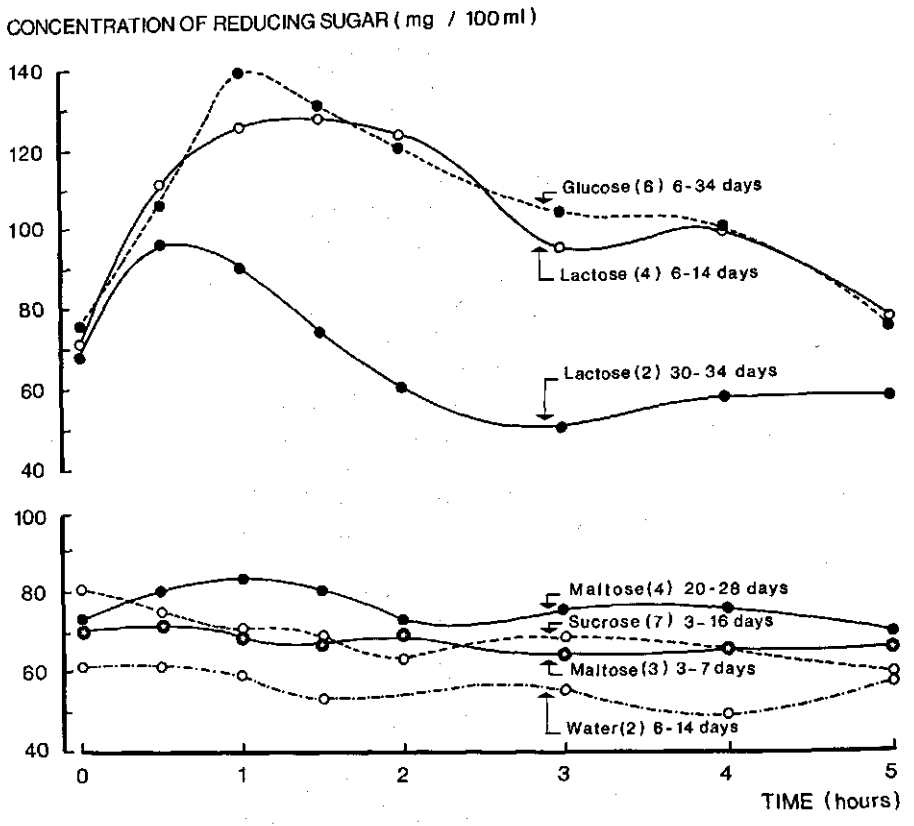

FIG. 6. Concentrations of reducing sugars (mg/100 ml) in the blood of calves given by mouth various carbohydrates at the rate of 2 g/lb. body weight. Mean values for stated numbers (in parenthesis) at given ages. (DOLLAR et al., 1957).

stimulated growth and kept faecal pH about normal. However, the growth still tended to be lower than that observed in the control (lactose) group. The diet without the enzymes caused the well-known adverse effects of starch induced scouring and weak performance. These results confirmed that both, low α-amylase and insufficient maltase activity in the young calf put strict limits on the utilization of oligosaccharides and starch in milk replacer diets.

Several authors emphasized a close relationship existing between the age of the animal and the activity of carbohydrate splitting enzymes (figure 6). The hexose absorption seems to be less evidently, or not at all affected by the age (65, 78, 162, 178, 184). A significant decline of the lactase activity in milk-fed calves is by many authors presumed to be the result of increasing age (23, 45, 79, 169, 178). Based upon their experimental results, neither GROPP (1973), nor COOMBE et al. (1974) could confirm that supposition. They supposed that the presumed relationship merely resulted from adaptive effects to dietary changes. In most experiments, indicating the relationship presumed, lactose intake per kg BW decreased to a similar degree with age as did the enzymatic activity of lactase.

Contrary to lactose, much evidence remains that age is involved in the utilization of starch or its derivatives in calves (23, 79, 85, 121, 169, 172, 176). Significant responses to starch intake were usually not observed before six weeks of age, even after a long period of adaption (26, 45, 55, 77, 78, 83, 84, 112, 126, 127, 129, 130). Amylolytic activity seems to increase steadily in older animals, although it remains lower than the lactase activity. It is not absolutely clear, whether or not this development can be stimulated by adapting the animals to gradually increased starch intake after five weeks of age.

4.4. Conclusions

The young, milk-fed calf has an impressive ability to digest lactose and absorb glucose and galactose. Consequently the levels of intake permitted in these animals are rather high. The digestion of other carbohydrates is much less developed or even lacking. Their dietary intake is therefore strictly limited or even not tolerated at all.

When the level of intake exceeds the tolerated one, scouring will occur. Bearing in mind the literature cited in Chpt. 2, the results seems to indicate that the digestive mechanisms involved in these disturbances may be not unlike to those described in infant fermentative diarrhoea.

The literature is less informative about the digestive changes in calf's intestine that are responsible for the scouring. Neither have the consequences of this disturbance for the water and mineral metabolism in calves been investigated systematically. The main objective of our work was to get more information about these aspects.

5. LACTOSE DIGESTIBILITY IN MILK-FED CALVES AND ITS RELATIONSHIP WITH FERMENTATIVE DIARRHOEA. EXPERIMENTAL RESULTS

5.1. INTRODUCTION

In respect of the relationship between high lactose intake and scouring in young calves, four important questions were neither clarified in the literature nor in our experiments described in Chpt. 3.

a. What dietary level of lactose is the maximal limit, still tolerable in regard to its disturbing effect on faecal consistency?
b. What changes in the dietary digestion and absorption can be expected in the small intestine when lactose intake exceeds this limit?
c. What is the relationship between the qualitative and quantitative parameters of the digesta flowing into the lower intestine and faecal characteristics?
d. What effect may be expected from lactose induced scouring in calves on the water and mineral excretion?

In a series of ten experiments (Exp. 6–15), involving 110 milk-fed calves we have tried to get more information to answer these questions.

ad. a. According to the literature and practical experience, scouring in calves will occur when lactose is fed in excess of the digestive and absorptive capacity of the small intestine. Quantitative data about the highest level tolerated are, however, hardly documented. Nor is it fully clarified, whether or not animals' age or digestive adaption to high lactose intakes may affect this limit to such an extent that it will have practical implication.

In two experiments (Exp. 6, 7) the effect of high dietary lactose intake on carbohydrate digestion, using the blood sugar response as parameter, and on scouring in calves was investigated. The results of these trials are described in section 5.3. In addition to this main item, the influence of age on calf's response to high lactose intake was investigated in Exp. 6. In Exp. 7 particular attention has been paid to the adaptive response in calves to high lactose intake, when treated for a longer time.

ad. b. Two phases can be distinguished in the digestive process of carbohydrates in the small intestine: the interluminal phase and the brush border phase, the latter reflecting the digestion and final absorption of the monosaccharides. If high lactose intakes are to disturb the normal digestive process, it can interfere in both phases. The effect on the interluminal phase may be related to changes in digesta composition and osmolality, and/or changes in digesta transit time in the small intestine. The second phase, digestion and absorption, was of interest in our work, because the literature was not conclusive as to whether or not the lactase activity is limiting for maximal lactose digestion and absorption.

In three subsequent trials (Exp. 9–11) the effect of varying lactose (or sucrose) intakes on the pattern of digesta flow, transit time and osmolality were the main

items investigated. In Exp. 12 the effect of high lactose intake on the apparent digestibility of dietary components was measured. A possible interference of diet composition in the digestive process under these conditions was tested as well. In two subsequent experiments (Exp. 13, 14) the significance of lactase activity in maximal lactose digestion and absorption was investigated. The results of these six experiments are reviewed in section 5.4.

ad. c. The eight experiments described above relied upon the generally accepted assumption that an increased flow of undigested carbohydrates into the lower intestine is responsible for dietary scouring (Chpt. 2). In a previous experiment (Exp. 5, Chpt. 3) this assumption was tested by infusing graded levels of lactose, up to 20% of oral NFE intake, into the lower intestine. The calves, however, responded remarkably mildly to those infusions by comparision with the results usually observed after high oral intakes. It was therefore decided to repeat this trial. Lactose and galactose were infused into the hind gut in an amount equal to that presumed to occur in scouring calves and the diarrhoeic effects were measured. The results of these experiments (Exp. 8, 11) are given in section 5.5.

ad. d. The literature provides much evidence that scouring calves may lose considerable amounts of water and minerals. The quantitative data on mineral losses given in the literature, however, were confined exclusively to calves suffering pathogenic infections. It was not clear whether or not these results were applicable in calves suffering fermentative diarrhoea. The effects of dietary lactose on the water and electrolyte excretion were investigated in Exp. 9, 14, 15 and Exp. 15, respectively. The results are described in section 5.6.

5.2. General experimental procedures

Most of the methods used in Exp. 6–15 are described in section 3.2 or summarized in the Appendix for each experiment. The experimental designs and composition of the diets are given in detail in the Appendix.

Each trial consisted of separate experimental periods, usually lasting 3 or 4 days. The animals were abruptly changed to the high carbohydrate diets at the start of each period and sampling procedures were started immediately. Between each experimental period usually 3–4 days were allowed for recovery of the calves. The animals received a control diet (diet A) in the recovery periods.

Based on the experiences in previous experiments, significant differences between individual calves and maybe between days of treatment could be expected in addition to, and often interfering with, the dietary differences, which were our main interest. Neither could seasonal effects be neglected. Calves born in springtime and early summer responded usually more seriously to the dietary treatments than those born at other times of the year. These effects were taken into consideration in the experimental designs. All samples were analysed individually, except were another procedure is indicated. Further, in each experiment a control group was included. These precautions enabled the statistical

evaluation, usually by F-test or multiple regression analysis, of the separate effects.

Experimental diets

The effect of dietary lactose intake was tested by using three separate treatments: A, B and C. These treatments were characterized by a different level of lactose intake, while the intakes of crude protein and ME were about the same. The quantity of lactose offered daily in treatments A, B and C was approximately 10, 13.5 and 17 g Hex. Eq. per kg BW, respectively.

Three diets were used, referred to as diet A, diet B and diet C, respectively. The chemical composition of (control) diet A was approximately similar to that of Dutch commercial milk replacers used for veal calves. The composition of diet B and C differed in their lactose content, which was increased progressively at the expense of fat and skim milk powder. In the respective treatments about 19.6 g diet A, 22.0 g diet B and 23.7 g diet C were fed per kg BW per day, to achieve the desired intake of Hex. Eq. with equal amounts of crude protein and ME. The dilution rate of diet A depended on age, but was usually 1:5.5; those of diets B and C were based on equal water intake in the three treatments. More detailed information about the diets used in the separate experiments are given in the Appendix.

In some experiments other diets were used to compare their diarrhoeic effect with that of the lactose diets. For that purpose diet D was tested in Exp. 10 and 11. This diet provided daily amounts of ca. 8 g Hex. Eq. as lactose and ca. 3 g Hex. Eq. as sucrose per kg BW. In Exp. 14 a milk substitute was used, containing almost exclusively free hexoses (diet C'). In this diet the lactose and skim milk powder in diet C were replaced by its hexoses and a skim milk powder, consisting mainly of hydrolysed lactose, respectively.

Sampling of blood

In Exp. 6 and 7 blood was sampled from the jugular vein to investigate the blood sugar response. Nine samples were collected from $\frac{1}{2}$ h pre-feeding until $7\frac{1}{2}$ or 9 h post-feeding. In Exp. 6 the blood was sampled manually; in Exp. 9 a permanent catheter was used. Each sample (ca. 5 ml) was mixed with approximately 1 mg NaF solution to prevent glycolysis and 20 mg heparin to prevent coagulation. Samples were kept frozen until analysed.

Chemical methods

In addition to the chemical methods given in section 3.2, the methods used for the carbohydrate analysis need further specification.

In most experiments the individual samples were analysed for reducing substances content; the number of samples was too high to analyse all of them for the individual carbohydrates. It was presumed that the reducing substances content would be reasonably representative of the carbohydrate content. Reducing substances were analysed according to BROWN et al. (1961) and the modifications proposed by BITTNER et al. (1963) on a Technicon Auto-analyser at 460

Nm. Glucose was used as a standard in blood and urine analyses; lactose served as a standard in all other samples.

The separate sugars, glucose, galactose and lactose, were analysed according to BOEHRINGER (1977). The method is based upon enzymatic phosphorylation of glucose and galactose, measuring the NaDPH and NaDP at 340 Nm spectrophotometrically. The results obtained in this enzymatic analysis showed that the reducing substances content was actually a useful parameter in samples of the diet, duodenal chyme and blood. The results obtained in samples of ileal chyme and faeces seemed, however, to disagree with the data obtained in the reducing sugar analysis. Other carbohydrate components, in particular consisting of galactose, made both analytical methods unsuitable for that material, as is described in detail in section 5.4.3.

To distinguish the separate sugars in these samples, the method described by OLLING (1972) was chosen. According to this method, trimethylsilylether-derivatives of the sugars are measured by gas chromatography.

The animals in general seemed to be healthy during the experimental periods, except for the digestive disturbances induced. In the event of an animal becoming diseased, it was withdrawn immediately from the experiment and the results obtained were excluded from consideration.

5.3. THE EFFECT OF DIETARY LACTOSE ON FAECAL CHARACTERISTICS AND BLOOD SUGAR RESPONSE

In Exp. 6 the effect of graded levels of dietary lactose on faecal characteristics was investigated, using thirty calves, allotted to five groups of six animals each (see Appendix, page 121). The individual faecal responses of the calves were measured and related to their blood sugar responses, presumed to represent the individual ability to digest and absorb lactose in the separate treatments. Diets A, B and C were fed to 3 'test' groups according to a latin square design with four replications. This design was chosen to permit the measurement of the effect of age on lactose tolerance, using the scouring response and blood sugar curve as parameters. The other two groups served as 'control' groups, to check a possible carry-over effect from the frequent changes in feeding regimes (see Appendix, page 121). The results proved, however, that no carry-over effect occurred.

The high dietary lactose intake in treatments B and C resulted in scouring after one or two feedings (table 12). Approximately 2.5 % of the samples collected in (control) treatment A were scored *Loose* or *Diarrhoeic*. Treatment B induced in total 9.4 and 4.5 % *Loose* and *Diarrhoea* scored faeces, respectively. The diarrhoeic property of treatment C was much more marked. Only 54 % of all samples collected in this treatment were judged to be *Normal*. Abnormal faecal consistency was only occasionally observed in treatment A, almost all excreted in the first experimental period (P_1), when the animals aged two weeks. Faecal pH did not suggest that lactose was primarily responsible for the reduced faecal con-

TABLE 12. The effect of the treatments A, B and C on faecal consistency score, pH and DM content (Exp. 6).

		Treatment A[1]	Treatment B	Treatment C
Actual daily intake (g Hex. Eq./kg BW)[2]		9.7 ±0.3[3]	13.3 ±0.6	16.2 ±0.8
Faecal characteristics:				
Visual score[4]	*N*	537	265	173
	L	17	29	71
	D	16	14	75
Faecal pH		6.9 ±0.7[a]	6.4 ±0.8[b]	5.6 ±1.1[c]
Faecal DM (%)		15.19 ±3.63[a]	12.79 ±3.88[b]	10.10 ±3.39[c]

[1] The samples collected in the control groups are included in the results of treatment A.
[2] Level of intake corrected for the reduced feeding schedule in P_1-P_3 (see Appendix, page 122).
[3] Mean ± sd; means not sharing a common letter differ significantly (F-test).
[4] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.

sistency in the control treatment. The acidity of the abnormal faeces in this group of calves averaged in P_1 5.8 ± 0.6 and 5.3 ± 0.8 for *Loose* and *Diarrhoea* scored samples, respectively. The pH in the *Normal* faeces averaged 5.6 ± 0.3 in the same period. In practice it is well known that calves are sensitive to scouring at that age, particularly because of pathogenic infection. Although rectal temperature of the scouring calves did not indicate such an infection, some pathogenic effect on the results cannot be excluded.

Faecal pH and DM content were lower in treatments B and C, compared with those in treatment A (table 13). These faecal parameters closely reflected the visual faecal score. The 1197 individual data, obtained in this experiment, showed that faecal pH averaged 6.7 ± 1.3, 5.3 ± 0.8 and 4.5 ± 0.7 in *N*, *L* and *D* scored samples, respectively. The corresponding means for the DM content were 14.0 ± 3.1, 8.9 ± 2.4 and 6.4 ± 1.9%.

Scouring frequency gradually decreased in the first three weeks of the experiment (P_1–P_3), as is shown in table 13. In this respect it has to be stressed that feed intake increased in these weeks from 55 to 90% of the intended level (see Appendix, page 122). The actual daily lactose intake increased from 5.5 tot 9.8, from 7.5 to 13.5 and from 9.4 to 17.0 g Hex. Eq. per kg BW in treatments A, B and C, respectively. The results in treatments B and C in these periods, showing improved faecal characteristics compared to P_1, seem to provide much evidence that calves adapt rather quickly to high lactose intakes at that age. In the subsequent periods scouring frequency remained fairly constant, untill in P_{11} and P_{12} when frequency increased again, particularly in treatments B and C. The effect of age on lactose tolerance seemed thus to be negligible from 4–10 week of age in this trial. Afterwards, calves' tolerance for lactose tended to decrease again.

However, the interpretation of the faecal response in P_{11} and P_{12} as an effect of age on lactose digestion and absorption was complicated by the feeding schedule. This schedule, a fixed quantity of feed per kg BW, differed from that

TABLE 13. The effect of the dietary treatments on faecal characteristics in each experimental period of Exp. 6.

Exp. per.	Treatment A			Treatment B			Treatment C		
	Abnormal faeces %[1]	pH	DM %	Abnormal faeces %	pH	DM %	Abnormal faeces %	pH	DM %
P_1	35	5.5 ±0.6[a2]	12.94 ±6.50[a]	60	5.3 ±1.0[a]	13.01 ±6.81[a]	100	4.6 ±0.4[b]	6.21 ±0.66[b]
P_2	5	6.5 ±0.6[a]	19.72 ±4.17[a]	42	6.0 ±1.0[b]	13.11 ±6.52[b]	77	4.7 ±0.7[c]	8.26 ±2.83[c]
P_3	0	6.8 ±0.5[a]	16.78 ±3.38[a]	22	6.0 ±1.1[b]	13.59 ±5.88[b]	61	5.7 ±1.0[c]	9.23 ±3.69[c]
P_4	0	7.0 ±0.5[a]	17.40 ±3.29[a]	0	6.3 ±0.6[b]	11.96 ±2.22[b]	24	5.2 ±0.9[c]	10.05 ±3.93[c]
P_5	0	6.9 ±0.6[a]	16.26 ±2.92[a]	0	6.4 ±0.6[b]	13.11 ±1.99[b]	37	5.4 ±0.9[c]	10.86 ±3.72[c]
P_6	0	6.9 ±0.4[a]	15.24 ±2.12[a]	0	6.7 ±0.6[a]	14.02 ±2.74[b]	25	6.1 ±1.0[b]	12.21 ±2.80[c]
P_7	0	7.0 ±0.6[a]	15.43 ±2.46[a]	0	6.5 ±0.6[b]	13.90 ±2.87[b]	25	5.5 ±1.0[c]	10.65 ±2.37[c]
P_8	0	7.0 ±0.4[a]	14.50 ±1.78[a]	0	6.8 ±0.6[b]	13.46 ±2.22[b]	50	5.8 ±1.0[c]	11.11 ±2.53[c]
P_9	0	7.3 ±0.4[a]	14.22 ±2.06[a]	0	6.8 ±0.4[b]	12.90 ±1.87[b]	28	6.3 ±1.3[b]	11.00 ±3.04[c]
P_{10}	0	7.2 ±0.4[a]	13.65 ±2.23[a]	4	6.8 ±0.6[b]	12.12 ±2.24[b]	14	6.3 ±0.9[c]	11.46 ±1.98[b]
P_{11}	7	7.3 ±0.4[a]	12.84 ±2.15[a]	0	6.4 ±0.7[b]	11.38 ±2.20[b]	44	5.8 ±1.2[c]	10.13 ±2.98[b]
P_{12}	4	7.2 ±0.5[a]	12.94 ±2.03[a]	33	6.6 ±0.8[b]	10.82 ±2.82[b]	75	5.3 ±1.2[c]	8.72 ±4.35[c]

[1] Number of faecal samples scored *Loose* and *Diarrhoea* as % of total number of samples.
[2] Mean ± sd; means not sharing a common letter differ significantly between treatments (F-test).

generally applied in practice which is largely related to calf's metabolic weight. It resulted in a relatively high feed intake at the end of the experiment and an increased frequency of dietary refusals in P_{11} and P_{12}. The scouring observed in these periods may have been partly the result of 'overfeeding', irrespective of lactose intake. The faecal parameters in the animals receiving treatment A in P_{11} and P_{12} seemed to reflect the effect of 'overfeeding'. The changes in faecal consistency and DM content were not accompanied by a decreased pH and seemed therefore not representative of lactose induced scouring. However, in treatments B and C faecal characteristics were actually representing fermentative diarrhoea. The faecal responses to these treatments did not, therefore, permit any firm conclusion as to whether or not age had affected the lactose tolerance after ten weeks of age.

The glucose levels in jugular blood, measured as reducing substances, responded quickly to the ingestion of the diets. They increased until a maximal level was reached at 2–2.5 h post-feeding. Thereafter blood sugar level declined again, until the initial level, measured at 0 h, was reached (table 14, figure 7).

The individual blood sugar data were used to calculate for each period the blood sugar curve in each animal, according to DUCHATEAU et al. (1972). For further statistical interpretation, the maximal blood sugar level (y_{max}), as well as the time at which that level was reached (x_{max}) were calculated by differentiation

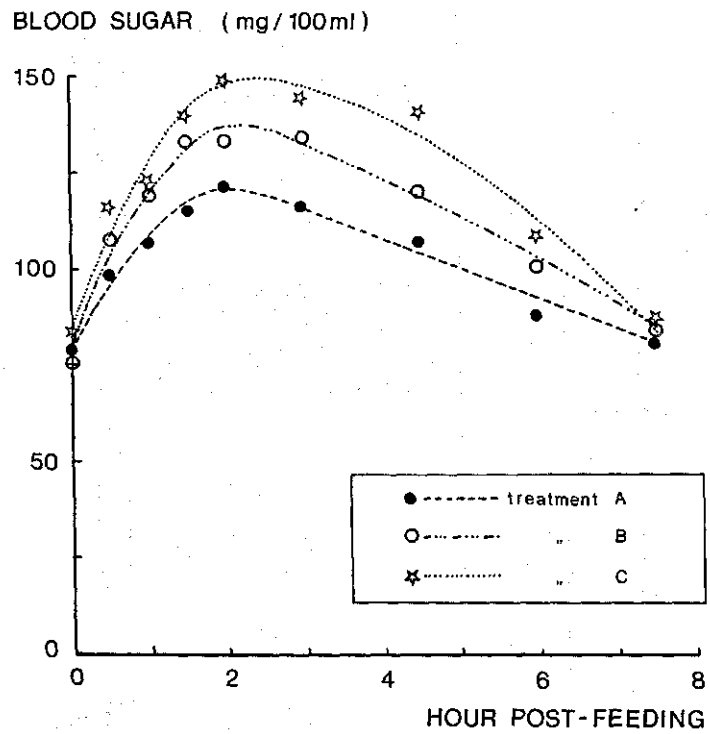

FIG. 7. The blood sugar response in calves, receiving treatment A, B or C (Exp. 6).

TABLE 14. The effect of treatments A, B and C on the blood sugar level in V. jugularis (Exp. 6).

Hour post-feeding	Blood sugar level (mg/100 ml blood)[1]		
	Treatment A	Treatment B	Treatment C
0	80 ±10[a 2]	76 ±13[a]	79 ±10[a]
0.5	99 ±15[a]	108 ±15[ab]	116 ±22[b]
1	107 ±20[a]	119 ±16[b]	123 ±24[b]
1.5	115 ±22[a]	133 ±22[b]	140 ±27[b]
2	121 ±24[a]	133 ±39[ab]	149 ±34[b]
3	116 ±26[a]	134 ±31[b]	144 ±33[b]
4.5	107 ±21[a]	120 ±28[a]	140 ±36[b]
6	88 ±18[a]	100 ±24[b]	108 ±24[b]
7.5	81 ±13[a]	85 ±28[a]	86 ±21[a]

[1] Reducing substances; glucose was used as reference standard.
[2] Mean ± sd; means not sharing a common letter differ significantly within time post-feeding (F-test).

of the smoothed curves (figure 8). Mean blood sugar level ($\bar{y}$), proving to be highly representative of the curve's surface ($r = 0.98$), was initially used as a third parameter for the individual curves. The results indicated, however, that both parameters, y_{max} and $\bar{y}$, were highly correlated within treatments and responded equally to the separate treatments A, B and C. Therefore either the one or the other was used in the further comparisons.

Maximal blood sugar level (y_{max}) was significantly higher on the higher lactose allowances (table 15). Statistical analysis (F-test) showed that the blood sugar level in V. jugularis increased significantly more from 0.5 to 6 h post-feeding when the calves received higher amounts of dietary lactose. It confirmed current

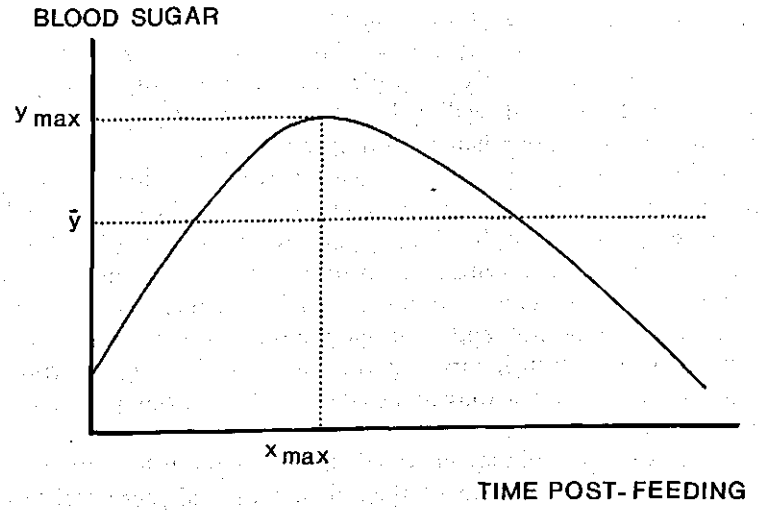

FIG. 8. Parameters used to estimate the blood sugar response.

TABLE 15. The response of some parameters from the blood sugar curves to treatments A, B and C (Exp. 6).

Parameter blood sugar curve in jugular vein[1]	Treatment		
	A	B	C
y_{max} (mg/100 ml blood)	120 ±18[a 2]	138 ±36[b]	149 ±34[c]
x_{max} (minutes)	127 ±40	131 ±41	140 ±34

[1] y_{max} represents the maximal blood sugar level, calculated from the data according to DUCHATEAU et al. (1972); x_{max} represents the time post-feeding at which y_{max} was reached.
[2] Mean ± sd; means not sharing a common letter differ significantly (F-test).

views that the lactose digestion is not primarily limited by lactase activity and, moreover, that the hexose absorption is not completely limited by saturation of the hexose absorption mechanisms (Chpt. 4). Only small and non-significant differences were observed in x_{max}. Maximal blood sugar level was thus established at approximately the same time post-feeding in all treatments. Maximal hexose absorption seemed to be attained at about 2–2.5 h post-feeding, irrespective of lactose intake.

These considerations are based on the assumption that the blood sugar curve was representative for the sugar absorption in this experiment. One has, however, to bear in mind that the curves may have underestimated actual blood hexose content, particularly in the treatments B and C. The preference for glucose in hexose absorption will presumably have stimulated the glucose absorption at the onset of lactose digestion and absorption. At the end of this process mainly galactose will have been absorbed. The galactose content in the jugular blood at that time may have been higher than is indicated by the reducing substances analysis, because the reducing activity of galactose is lower than that of the glucose reference.

Whether or not the blood sugar response was affected by age, was investigated by multiple regression analysis (DANIEL and WOOD, 1971). Based upon 141 individual curve parameters, it was concluded that the blood sugar content measured from 1–4.5 h post-feeding was higher when the calves grew older, irrespective of lactose intake. However, the initial concentration at 0 h and that measured at 6 and 7.5 h post-feeding decreased significantly with the age of the animals. These results strengthened our impression that the blood sugar curve became more 'compressed' post-feeding when calves grew older.

Contrary to the results on faecal characteristics, blood sugar levels did not indicate any effect of age on the blood sugar response at the end of the experiment, i.e. in P_{11} and P_{12}. It confirmed our supposition that the animals suffered 'overfeeding' in these periods, resulting in a higher frequency of scouring. The experimental design did not lend itself making the distinction whether, and to which extent, lactose transit time, lactose digestion, hexose absorption rate and/or glucose clearance rate were involved in the changes observed in the blood sugar curves when calves grew older.

Similar statistical techniques provided the opportunity to compare the individual blood sugar responses with the individual faecal characteristics of the calves during the same experimental period. This aspect was thought to be of

interest, because the differences between the individual responses tended to increase with lactose intake. For that reason the average faecal pH and DM content of each calf and in each experimental period was compared with the individual blood sugar response (y_{max}) on the first day of that period. Low, but significant correlations were found between the faecal parameters and the maximal blood sugar content ($r = +0.44$ and $+0.33$ for faecal pH and DM content, respectively). These positive correlations provide some evidence that the animals, being more susceptible to fermentative diarrhoea, are less efficient in lactose digestion and absorption. The fact that the blood sugar response at day 1 was compared with the average faecal pH and DM content, measured over four days, may have been partly responsible for the rather low correlation coefficients.

In this experiment faecal consistency declined significantly in the treatments B and C after the first experimental day, as did the quantitative parameters, pH and DM content. The higher values for faecal characteristics on the first experimental day seem to be rather normal. A considerable amount of the faeces collected at that time will have originated from control diet A fed in the pre-experimental or recovery period. The sampling periods (3 days) were too short to allow us to determine the extent to which the calves had adapted, or might adapt later to continued high lactose intakes, as reported in the literature. The blood sugar data were not informative on this aspect, as they were only measured on the first day of treatment.

Exp. 7 was specially designed to measure whether or not calves' response to high lactose intakes would change when the treatment lasted for more than three days. The period chosen was seven days, because we considered this would be long enough to reveal any effect that would be of practical importance. Ten calves, fitted with permanent catheters in the jugular vein, were used for that purpose (see Appendix, page 123). They were allotted to two groups and received treatment A or C, according to a change-over design. Each experimental period was prolonged to seven days in this experiment. The individual blood samples were collected at day 1, 4 and 7, just before and after the morning feeding.

The main results of Exp. 7 are summarized in table 16. Treatment C readily induced scouring as was expected. The mean faecal pH was about equal to those observed in Exp. 6 in animals of comparable age and treatment. The maximal, as well as the average blood sugar level was similarly increased in treatment C as was also found in the preceding trial. However, contrary to the experiences in Exp. 6, maximal blood sugar level was achieved significantly later when diet C was fed. In these calves x_{max} was retarded by about 90 minutes. The reason for this delay, which was observed only in this experiment, is not known.

The results showed again no adaptive effects in faecal response (table 17). Faecal pH was about constant during the experimental period on control diet A. The faeces were about normal on the first day of treatment C but had a lower consistency afterwards. The faecal results provided no evidence that adaptation to high lactose intake occurred in the second part of the experimental periods.

The blood sugar responses also suggested that no adaptive effect on lactose

TABLE 16. The effect of dietary lactose on faecal characteristics and blood sugar parameters (Exp. 7).

		Treatment A	Treatment C
Number of calves		10	10
Actual daily intake (g Hex. Eq./kg BW)		9.9 ± 0.4[1]	16.4 ± 1.4
Faecal characteristics:			
Visual score[2]	*N*	52	66
	L	–	13
	D	–	4
pH		7.3 ± 0.7[a]	6.0 ± 0.9[b]
Blood sugar parameters:[3]			
$\bar{y}$	(mg/100 ml blood)	102.3 ±10.0[a]	135.9 ±15.2[b]
y_{max}	(mg/100 ml blood)	134.3 ±20.0[a]	167.9 ±24.0[b]
x_{max}	(minutes)	135 ±42 [a]	226 ±55 [b]

[1] Mean ± sd; means not sharing a common letter differ significantly (mult. regression analysis).
[2] Number of samples scored *Normal (N), Loose (L)* or *Diarrhoea (D)*.
[3] See for y_{max} table 15. $\bar{y}$ represents the average blood sugar level, calculated according to DUCHATEAU et al. (1972).

digestion and absorption occurred in seven days of treatment. Although in both treatments significant differences between the mean blood sugar levels ($\bar{y}$) were measured on the separate days, these differences were only small within the treatments. This was especially so when these differences were compared with those observed in preliminary experiments, where diet A was fed to young calves and the daily variation in calf's blood sugar response proved to be approximately 10%. Neither did treatment C show any systematic effect of adaptation in Exp. 7 when lasting for seven days.

Summarizing the results obtained in Exp. 6 and 7 we concluded that fermentative scouring occurs when the daily lactose intake exceeds 10 g Hex. Eq. per kg BW in calves aged at least 4 weeks. The response in blood sugar level seems to indicate that lactose digestion and absorption will still increase when that dietary

TABLE 17. Mean faecal pH and blood sugar parameters on the separate days of treatment (Exp. 7).

Experimental day	Faecal pH		Mean blood sugar level ($\bar{y}$; mg/100 ml blood)[1]	
	Treatment A	Treatment C	Treatment A	Treatment C
1	7.0 ±0.8[2]	7.0 ±1.2[a]	105.2 ±10.8[a]	129.0 ±17.1[a]
2	7.0 ±1.3	5.6 ±0.8[b]		
3	7.3 ±0.4	5.7 ±0.9[b]		
4	7.6 ±0.5	6.2 ±0.6[ab]	103.8 ± 6.5[ab]	143.4 ±14.1[b]
5	7.4 ±0.6	6.1 ±0.8[ab]		
6	7.6 ±0.6	5.8 ±0.6[b]		
7	7.5 ±0.7	6.2 ±0.8[ab]	97.6 ±11.9[b]	135.3 ±11.5[ab]

[1] See table 16.
[2] Mean ± sd; means not sharing a common letter differ significantly within treatments (F-test).

level is exceeded. Treatment C, allowing about 16–17 g Hex. Eq. per kg BW per day, resulted in significantly higher blood sugar levels than did treatment B, providing approximately 12.5–13.5 g Hex. Eq. per kg BW. Both treatments increased the blood sugar levels significantly in relation to control treatment A, providing about 9–10 g Hex. Eq. per kg BW per day.

Further, the results showed that calf's response to high lactose intakes did not change when the treatment lasted seven days. Only in their first 3 to 4 weeks of life young calves seem to increase remarkably their ability to digest and absorb lactose, resulting in a considerable increase in the maximal limit tolerated at that age.

No effect of age on maximal lactose digestion and absorption could be established from 4 to 12 weeks of age. The absorptive processes involved seemed, however, to be slightly 'compressed' post-feeding.

5.4. The effect of dietary lactose on the flow rate of digesta, transit time of dietary components and their apparent digestibility in the small intestine

In the literature intestinal digesta flow is not well defined in the terminology used. In this work we have characterized the digesta flow at the duodenal and ileal site of the small intestine by the terms: *cumulative flow*, *recovery*, *flow rate* and *transit time*.

The term *cumulative flow* of digesta (component) is used to express the total weight of wet digesta, or one of its chemical components, collected between the time of feeding and sampling. *Recovery* is used for the cumulative flow between two successive feedings and expressed as a percentage of oral intake with the preceding feeding. The *flow rate* on a specific time post-feeding expresses the weight of digesta (component) collected per unit of time in percent of the preceding oral intake.

The *duodenal flow rate* is expressed per *5 minutes*, using cumulative flow data. E.g. the duodenal flow rate of wet digesta at 3 h post-feeding is calculated according to:

$$\frac{(\text{cum. duod. flow of wet digesta at } 3\tfrac{1}{4}\text{ h} - \text{cum. duod. flow of wet digesta at } 2\tfrac{3}{4}\text{ h}) \times 100}{\text{total weight of liquid milk intake} \times 6^{1}}$$

[1] The time between the two cumulative flow measurements is 30 minutes = 6 × 5 min. (flow rate is expressed per 5 min.).

The *ileal flow rate* is calculated per *hour* as the average from the amounts collected during two successive sampling hours. The flow rates of digesta components are estimated in a similar manner.

The term *transit time* indicates the time between milk (component) intake and its passage at the intestinal lumen site sampled.

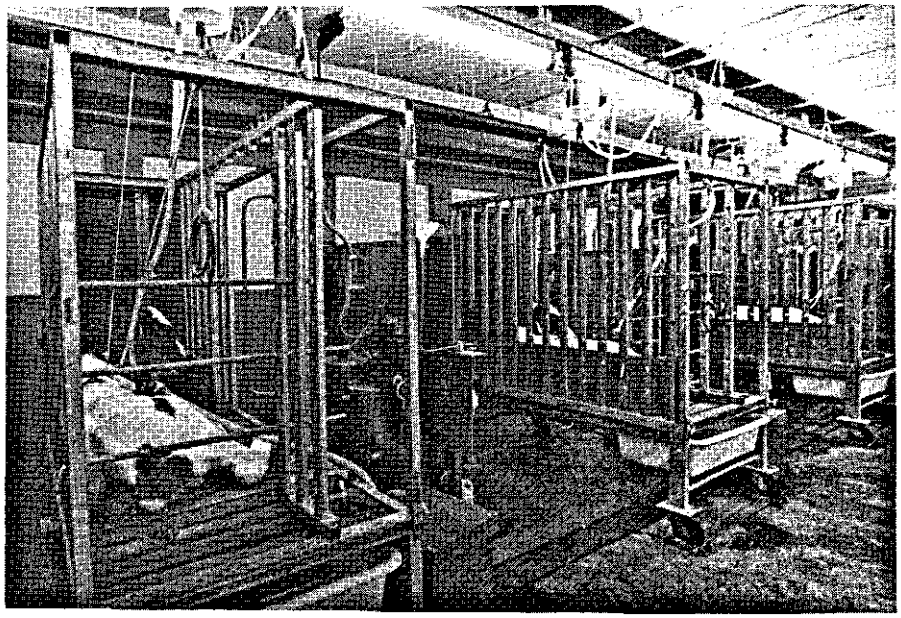

FIG. 9. Sampling duodenal and ileal digesta in calves.

5.4.1. *The effect of dietary carbohydrates on the digesta flow in the small intestine*

Exp. 9 was particularly designed to measure the effect of high dietary lactose on the digesta flow through the small intestinal lumen. In this trial sixteen calves were used, of which eight animals were fitted with re-entrant duodenal cannulae and four calves with re-entrant ileal cannulae. The collection of the intestinal digesta during the experimental periods prevented an accurate investigation of the scouring effect of the diets in the fistulated calves. Therefore, four non-fistulated calves served as a 'control group' to test the scouring property of high lactose intake in this experiment (see Appendix, page 125). The animals of each group were allotted to treatments A and C, according to a change-over design

TABLE 18. The diarrhoeic properties of treatments A and C in Exp. 9.

	Treatment A	Treatment C
Number of animals	4	4
Actual daily intake (g Hex. Eq./kg BW)	9.6 ± 0.6[1]	16.3 ± 0.8
Faecal characteristics:		
total excretion (g/12h)	104 ±80[a]	325 ±312[b]
visual score[2] *N*	38	44
L/D	–	42
pH	8.0 ± 0.4[a]	6.6 ± 1.0[b]
DM (%)	14.5 ± 2.4[a]	11.9 ± 3.9[b]

[1] Mean ± sd; means not sharing a common letter differ significantly between treatments (F-test).
[2] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.

TABLE 19. The flow rate of duodenal digesta in treatments A and C (Exp. 9).

Time post-feeding (h)	Flow rate[1]					
	Treatment A			Treatment C		
	Wet digesta	Nitrogen	Reducing substances[2]	Wet digesta	Nitrogen	Reducing substances
.125	3.25 ± 1.38[3]	2.20 ±1.09	1.64 ±1.01	3.26 ± 1.14	2.44 ±1.14	1.71 ±0.89
.25	2.25 ± 0.50	1.29 ±0.30	1.53 ±0.53	2.75 ± 0.65	1.71 ±0.56	2.06 ±0.69
.5	1.68 ± 0.40	0.84 ±0.21	1.25 ±0.44	2.14 ± 0.72	0.91 ±0.23	1.71 ±0.62
.75	1.64 ± 0.31	0.77 ±0.15[a]	1.20 ±0.30	1.51 ± 0.35	0.60 ±0.11[b]	1.14 ±0.25
1	1.86 ± 0.14[a]	0.82 ±0.14[a]	1.31 ±0.21[a]	1.49 ± 0.33[b]	0.61 ±0.14[b]	1.05 ±0.20[b]
1.5	1.71 ± 0.34	0.86 ±0.13	1.22 ±0.19	1.89 ± 0.59	0.78 ±0.22	1.34 ±0.37
2	1.69 ± 0.33	0.88 ±0.19	1.19 ±0.25	1.84 ± 0.48	0.82 ±0.16	1.24 ±0.33
3	1.74 ± 0.37	0.86 ±0.11	1.21 ±0.21	1.77 ± 0.31	0.81 ±0.13	1.08 ±0.19
4	1.51 ± 0.15	0.75 ±0.16	0.93 ±0.14	1.47 ± 0.15	0.73 ±0.14	0.86 ±0.13
5	1.34 ± 0.26	0.75 ±0.17	0.74 ±0.13	1.34 ± 0.20	0.72 ±0.11	0.69 ±0.16
6	1.17 ± 0.21	0.79 ±0.17	0.54 ±0.11	1.06 ± 0.25	0.69 ±0.12	0.47 ±0.15
7	0.90 ± 0.14	0.74 ±0.14	0.32 ±0.08	0.94 ± 0.22	0.73 ±0.16	0.32 ±0.11
8	0.79 ± 0.09	0.72 ±0.10	0.20 ±0.03	0.73 ± 0.15	0.63 ±0.12	0.17 ±0.06
9	0.69 ± 0.14	0.65 ±0.15	0.13 ±0.04	0.65 ± 0.15	0.58 ±0.10	0.12 ±0.05
10	0.71 ± 0.12[a]	0.63 ±0.09[a]	0.10 ±0.03	0.57 ± 0.13[b]	0.50 ±0.10[b]	0.07 ±0.03
11	0.59 ± 0.12	0.56 ±0.11	0.06 ±0.02	0.53 ± 0.13	0.48 ±0.13	0.05 ±0.02
12	0.44 ± 0.22	0.68 ±0.17	0.07 ±0.04	0.40 ± 0.21	0.60 ±0.25	0.06 ±0.04
Recovery[1]	167.6 ±11.1	109.0 ±8.1[a]	86.8 ±2.2[a]	162.8 ±12.7	99.6 ±8.1[b]	81.5 ±3.0[b]

[1] See page 51.

[2] Reducing substances measured against lactose as reference standard.

[3] Mean ± sd. Each mean is based on 24 observations. The actual daily intake averaged 9.1 ±1.7 and 15.7 ±0.8 g Hex. Eq./kg BW in treatment A and C, respectively. Means not sharing a common letter differ significantly between treatments (F-test).

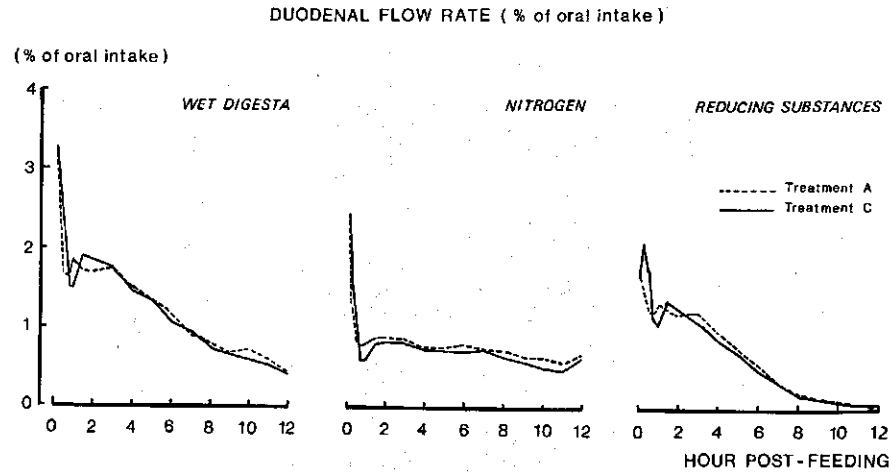

FIG. 10. The effect of dietary lactose on the average flow rates of duodenal wet digesta, nitrogen and reducing substances (Exp. 9).

with one replication.

Treatment A resulted in normal faecal characteristics in the non-fistulated 'control group' (table 18). Treatment C induced severe scouring in these animals; about 50% of the samples were scored *Loose* or *Diarrhoea*. Faecal pH and DM content were significantly lower than those observed in treatment A and total faecal excretion was higher. This higher quantity was mainly caused by an increased water excretion, although total DM excretion was increased as well. The response of the animals was as expected, although average faecal pH in both treatments was slightly higher than observed in Exp. 6 and 7.

The duodenal flow rates of wet digesta did not differ substantially between

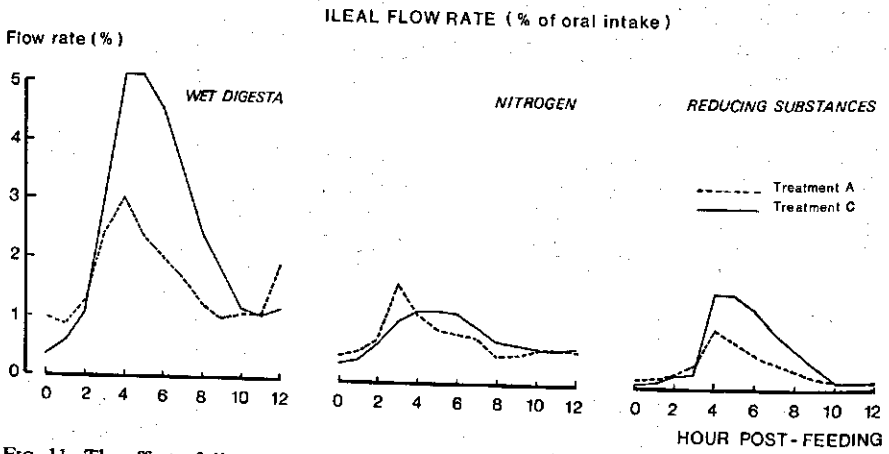

FIG. 11. The effect of dietary lactose on the average flow rates of ileal wet digesta, nitrogen and reducing substances (Exp. 9).

either treatment, nor between the experimental periods P_1/P_2 and P_3/P_4, although four other calves were used in the latter periods. The differences, occasionally observed, were only small and presumably not important (table 19, figure 10). The flow rates of the digesta constituents (nitrogen and reducing substances) did not indicate any disturbance in the duodenal flow. All curves reflected those usually observed in milk-fed calves (Ash, 1964; Hof et al., in press). High lactose intake seems not to affect the duodenal flow to such an extent that it interferes in the digestion and absorption of dietary components.

The difference between both treatments was more marked at the ileal site of the small intestine (table 20, figure 11). The flow rates of wet digesta increased in both treatments from approximately 1 h until 4 h post-feeding and declined afterwards. The maximal flow rate of wet digesta, achieved in both treatments at 4–5 h post-feeding, averaged about 3 and 5% of the oral liquid milk intake in treatments A and C, respectively. The latter treatment resulted in significantly higher flow rates than treatment A from 4 until 8 h post-feeding. During 12 h sampling the cumulative flow of wet digesta was about twice as high in treatment C as the weight collected in treatment A; wet digesta recoveries were 30.4 and 19.5% of oral liquid milk intake, respectively.

Neither nitrogen flow rates, nor nitrogen recovery at the ileal site was greatly affected by treatment C. Only slight differences existed between both diets from 5–7 h post-feeding. As the nitrogen intake was almost equal in both treatments, these results indicated that the amount of dietary protein apparently digested in the small intestine was not changed in treatment C.

The flow rates of reducing substances reflected those of wet digesta, suggesting a close relationship between the carbohydrate flow into the hind gut and the volume of ileal digesta. The maximal flow rate of reducing substances was reached at the same moment in both treatments. Obviously, the transit time of the carbohydrate fraction is not greatly affected by higher intakes of lactose.

From the actual amounts of reducing substances ingested, averaging 604.4 g and 981.2 g per feed in treatments A and C, respectively, 577.8 g (95.6%) and 904.7 g (92.2%) were apparently digested in the small intestine. The absolute amount of lactose digested and absorbed seemed thus to be considerably higher in treatment C than in the control. This result agreed with the significantly higher blood sugar response observed in Exp. 6 and 7 in the same treatment. However, the increase in the carbohydrate absorption in treatment C could not prevent about three times more reducing substances entering the hind gut than when diet A was fed in this experiment.

Statistical analyses (F-test) did not indicate any systematic difference between the experimental days on which the flow rates and the recovery of wet digesta or its components, nitrogen and reducing substances, were determined. The apparent digestibility in the small intestine was not affected by digestive changes in the small intestine of the calves when an excess of lactose was fed. Similar observations were obtained in the preceding Exp. 7.

The individual differences in response were more evident in treatment C than in treatment A, resulting in higher coefficients of variation in the former treat-

TABLE 20. The flow rate of ileal digesta in treatments A and C (Exp. 9).

Time post-feeding (h)	Flow rate[1]					
	Treatment A			Treatment C		
	Wet digesta	Nitrogen	Reducing substances[2]	Wet digesta	Nitrogen	Reducing substances
0	0.99 ±0.47[3]	0.46 ±0.30	0.11 ±0.12	0.35 ± 0.21	0.32 ±0.24	0.06 ±0.99
1	0.86 ±0.42[a]	0.51 ±0.23[a]	0.13 ±0.14	0.57 ± 0.34[b]	0.38 ±0.23[b]	0.07 ±0.06
2	1.26 ±0.79	0.73 ±0.40	0.22 ±0.23	1.05 ± 0.75	0.65 ±0.39	0.20 ±0.26
3	2.40 ±1.16	1.68 ±0.45	1.03 ±0.24	3.19 ± 2.66	1.01 ±0.52	0.55 ±0.25
4	3.01 ±1.11[a]	1.16 ±0.36	0.97 ±0.47	5.11 ± 3.66[b]	1.21 ±0.45	1.56 ±1.52
5	2.37 ±1.02[a]	0.90 ±0.35[a]	0.76 ±0.44[a]	5.11 ± 3.24[b]	1.23 ±0.32[b]	1.53 ±1.27[b]
6	2.01 ±0.93[a]	0.83 ±0.27[a]	0.55 ±0.25[a]	4.55 ± 2.43[b]	1.18 ±0.35[b]	1.32 ±0.95[b]
7	1.79 ±0.71[a]	0.76 ±0.22[a]	0.41 ±0.21[a]	3.48 ± 1.69[b]	0.98 ±0.30[b]	0.93 ±0.63[b]
8	1.24 ±0.66[a]	0.47 ±0.29	0.27 ±0.17[a]	2.45 ± 1.29[b]	0.71 ±0.33	0.64 ±0.42[b]
9	1.01 ±0.66[a]	0.47 ±0.24	0.15 ±0.09[a]	1.79 ± 1.13[b]	0.67 ±0.33	0.36 ±0.30[b]
10	1.08 ±0.66	0.55 ±0.27	0.10 ±0.08	1.19 ± 0.45	0.60 ±0.24	0.11 ±0.08
11	1.06 ±0.52	0.58 ±0.29	0.09 ±0.04	1.06 ± 0.52	0.55 ±0.25	0.09 ±0.05
12	1.96 ±0.76	0.56 ±0.49	0.08 ±0.09	1.19 ± 0.78	0.61 ±0.38	0.10 ±0.08
Recovery[1]	19.5 ±5.2[a]	8.6 ±1.6	4.4 ±1.3[a]	30.4 ±14.8[b]	9.7 ±2.2	7.8 ±5.7[b]

[1] See page 51.
[2] Reducing substances measured against lactose as reference standard.
[3] Mean ± sd. Each mean is based on 24 observations. The actual daily intake averaged 9.5 ±0.6 and 15.6 ±1.7 g Hex. Eq./kg BW in the treatments A and C, respectively. Means not sharing a common letter differ significantly between treatments (t-test).

ment. The individual differences observed at the end of the small intestine may have been indicative for those in the calf's susceptibility to lactose induced scouring. If so, it should validate the relationship observed between the individual blood sugar and faecal responses in Exp. 6.

The results of Exp. 9 proved to be quite characteristic of the effect of excessive carbohydrate intake on ileal wet digesta flow. In all experiments when ileal digesta were collected similar shifts in flow rate and recovery were observed when the carbohydrate intake increased. In table 21 the average flow rates are summarized, calculated from the data obtained in some preliminary experiments and from the result in Exp. 9, 10, 11 and 13 (see Appendix). Each mean value represents 126, 115 and 19 observations in the lactose treatments A and C and the sucrose treatment D, respectively. In treatment C the flow rates were significantly higher than in treatment A from 2 h until 10 h post-feeding. The moment at which the maximal flow rate was achieved was not very different in the two lactose treatments and averaged 4.9 ± 0.9 h and 5.1 ± 1.1 h post-feeding in treatments A and C, respectively. Treatment D, supplying 8 g Hex. Eq. lactose and 3 g Hex. Eq. sucrose per kg BW, induced significantly higher flow rates of wet digesta than did the high lactose treatment (figure 12). When sucrose was fed, total recovery of wet digesta averaged about 50 % of oral liquid milk intake at the

TABLE 21. The mean flow rates of ileal wet digesta in treatments A, C and D.

	Flow rate[1]		
	Treatment A	Treatment C	Treatment D
Number of animals	36	33	7
Actual daily intake (g Hex. Eq./kg BW):			
lactose	9.2 ±0.6[2]	15.9 ± 1.3	7.9 ±1.3
sucrose	–	–	2.8 ±0.2
Time post-feeding (h)			
0	0.79 ±0.49	0.88 ± 0.58	1.03 ±0.49
1	0.81 ±0.38[a]	0.91 ± 0.49[a]	2.60 ±1.57[b]
2	0.98 ±0.48[a]	1.62 ± 1.07[b]	5.83 ±3.12[c]
3	1.73 ±0.83[a]	3.33 ± 2.18[b]	8.34 ±2.90[c]
4	2.80 ±0.88[a]	4.85 ± 2.84[b]	8.51 ±2.35[c]
5	2.89 ±0.80[a]	5.00 ± 2.54[b]	7.09 ±1.62[c]
6	2.33 ±0.98[a]	4.27 ± 1.69[b]	5.19 ±1.47[c]
7	1.82 ±0.90[a]	3.34 ± 1.03[b]	3.81 ±1.39[c]
8	1.29 ±0.54[a]	2.44 ± 0.79[b]	3.71 ±1.10[c]
9	1.05 ±0.38[a]	1.78 ± 0.64[b]	3.19 ±0.85[c]
10	1.04 ±0.38[a]	1.34 ± 0.56[b]	2.23 ±0.56[c]
11	1.11 ±0.48[a]	1.21 ± 0.42[a]	1.77 ±0.66[b]
12	1.13 ±0.62	1.24 ± 0.45	1.64 ±0.73
Recovery[1]	18.4 ±5.1[a]	31.0 ±11.1[b]	53.9 ±9.2[c]

[1] See page 51.
[2] Mean ± sd; means not sharing a common letter differ significantly between treatments (F-test).

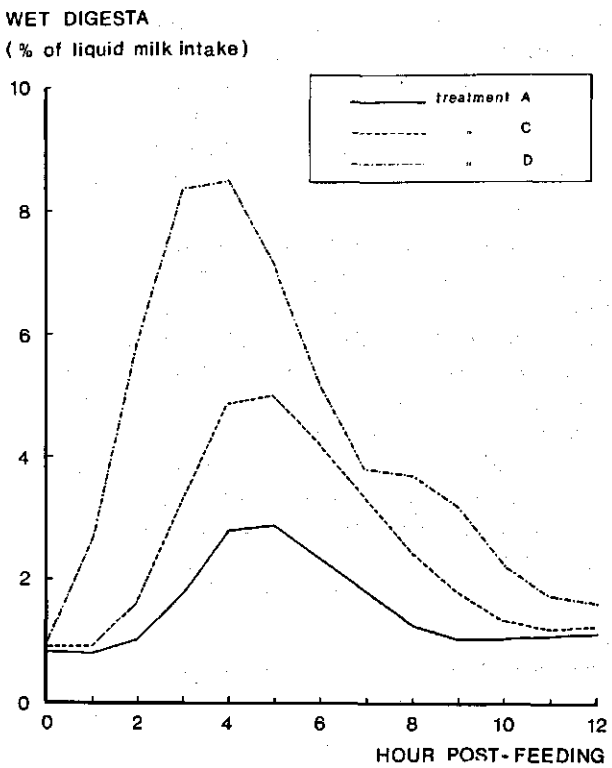

FIG. 12. The effect of treatments A, C and D on the average flow rates of ileal wet digesta.

end of the small intestine. Digesta transit time in abomasum and small intestine is decreased when sucrose is given to calves. The time at which maximal flow rate was reached in this treatment averaged 3.9 $\pm$ 0.7 h post-feeding, which was about 1 h earlier than in the lactose treatments. Sucrose seems thus to accelerate the flow of wet digesta through the digestive tract. The higher amount of osmotic active solutes, i.e. sucrose, in the lumen of the lower part of the small intestine results not only in an increased wet digesta weight, but seems also to be responsible for an accelerated digesta flow.

In the literature lactic acid is frequently reported as to be chiefly responsible for increased peristaltic movements of the intestine, which would decrease digesta transit time in fermentative diarrhoea. It is unlikely that in our experiments this end product of the saccharolytic fermentation has acted as a stimulus for digesta passage through the small intestinal lumen. A systematic investigation of the FFA content in ileal digesta in Exp. 11 (see Appendix, page 127), provided much evidence for that conclusion. The amount of lactic acid recovered at the ileal site of the small intestine was scarcely increased when treatment A was replaced by treatment C. The average formic, acetic and d, l-lactic acid content in ileal digesta were 60, 75 and 3 mg per 100 ml, respectively, in treatment A and 69, 56 and 7 mg per 100 ml in treatment C. Although we did not investigate the fatty

acid content in ileal digesta when sucrose was fed, ileal digesta pH did not indicate any difference in fermentation rate in the small intestine compared with lactose. The pH, measured as a routine in all experiments, was slightly alkaline on the sucrose treatment and did not differ from that on lactose feeding.

Summarizing the results of these experiments, we concluded that scours inducing levels of lactose do not affect either abomasal transit time, or duodenal flow rate of wet digesta, nor that of its components, nitrogen and reducing substances. At the ileal site neither digesta transit time, nor the flow rate of nitrogen is influenced. The flow rates of wet digesta and reducing substances are, however, substantially higher when lactose intake increases. Althought in treatment C the amount of sugar apparently digested and absorbed in the small intestine increases considerably, the quantity flowing into the hind gut is higher than in control treatment A.

Treatment D, supplying 2.3 – 3 g Hex. Eq. sucrose and 8 – 9 g Hex. Eq. lactose per kg BW per day, seems to act in scouring in a similar way as do high amounts of lactose (16–17 g Hex. Eq. per kg BW per day), although the response of the calves to the sucrose treatment is more serious. In relation to the lactose diets, wet digesta flow rate into the lower intestine is increased in the sucrose treatment and the digesta transit time in abomasum and small intestine seems to be reduced.

5.4.2. *The effect of dietary lactose on the apparent digestibility of dietary components in the small intestine*

The results, cited in section 5.4.1, suggest that the apparent digestibility in the small intestine of reducing substances decreases at higher levels of lactose intake. In these experiments ileal digesta were only collected from 8.00–20.00 h for three or four days. That might have been too short a time for measuring accurately enough the apparent digestibility in the small intestine. Moreover, the results obtained in Exp. 1, when feeding additional amounts of lactose or milk replacer, suggested an interaction between diet composition and lactose in the scouring response. We were interested, whether this interaction occurs in the small intestine or is caused by a difference in microbial fermentation in the hind gut. The objective in Exp. 12 was to measure both aspects (see Appendix, page 129).

Fifteen calves, fitted with re-entrant ileal cannulae, were allotted over three experimental groups, receiving either diet A, B or C in five experimental periods of five days. The effect of lactose intake on the apparent digestibility in the small intestine was measured in P_1 and P_5 by collecting ileal digesta for 5 × 24 h in each period. In these periods the groups received either treatment A (9 g Hex. Eq. per kg BW), treatment B (12.2 g Hex. Eq. per BW), or treatment C (15.3 g Hex. Eq. per kg BW). The interaction between lactose intake and diet composition in the apparent digestibility was investigated in $P_2 - P_4$. In P_2 all animals received 12.2 g Hex. Eq. lactose per kg BW per day in each of the three diets, increasing the daily allowance of diet A offered to group I by 35.5% and decreasing that of diet C offered to group III by 20% compared with the levels used in treatments A and

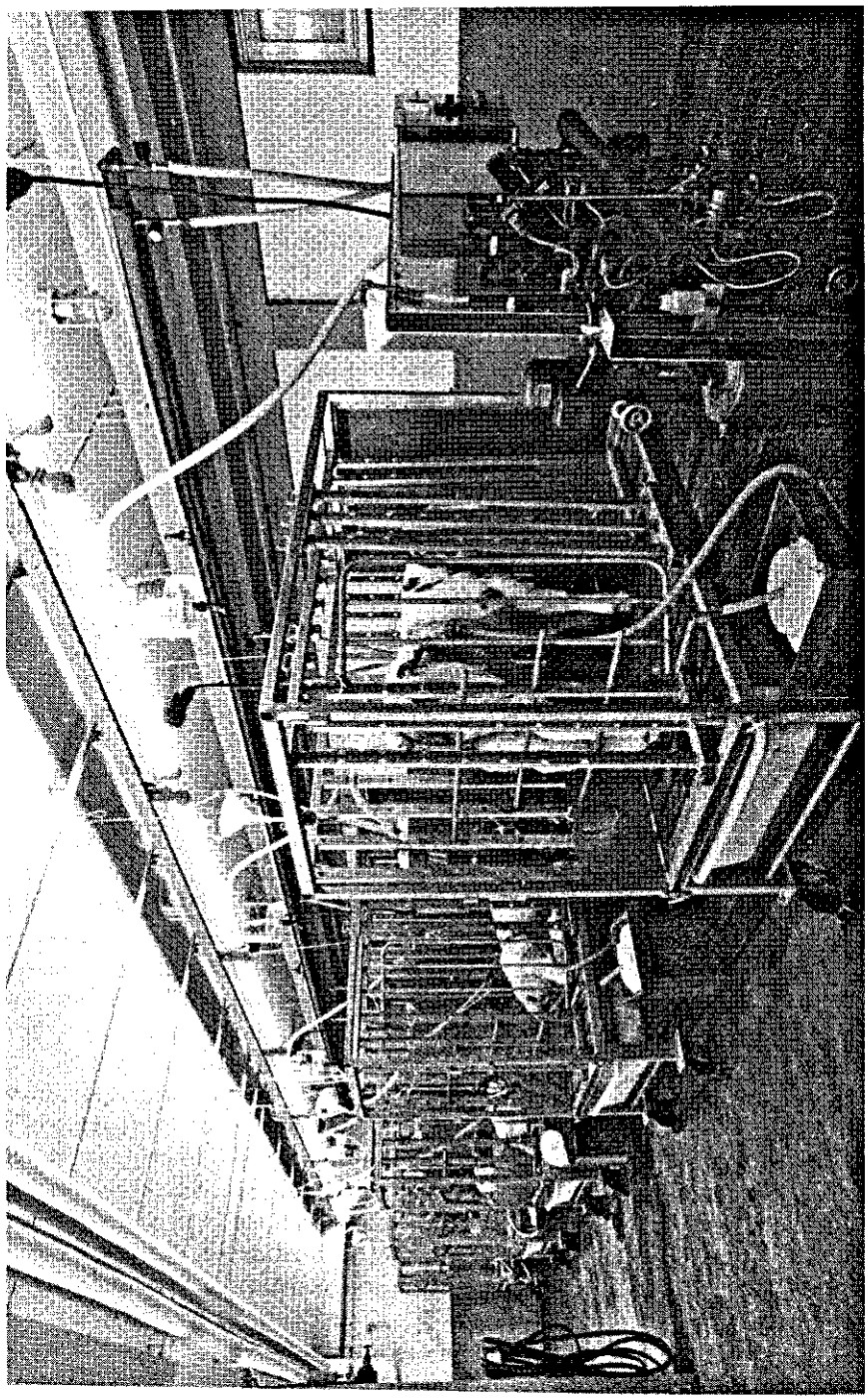

FIG. 13. Sampling ileal digesta in calves.

TABLE 22. The apparent digestibility of diet components in the small intestine (Exp. 12).

	Diet	Actual daily intake (g Hex. Eq./kg BW)	Apparent digestibility (%)[1]					
			DM	OM	N	EE	NFE	Reducing substances[2]
P_1/P_5	A	8.0 ±0.6[a 3]	92.7 ±1.3[a]	93.4 ±1.0[a]	91.3 ±1.9[a]	98.1 ±1.6	92.5 ±1.3[a]	94.6 ±1.6[a]
	B	11.2 ±0.5[b]	93.0 ±1.0[a]	93.5 ±1.0[a]	92.3 ±1.3[a]	98.6 ±0.7	93.0 ±1.1[a]	93.9 ±1.1[a]
	C	14.2 ±0.6[c]	90.2 ±1.2[b]	91.0 ±1.1[b]	89.6 ±1.8[b]	97.6 ±2.6	91.0 ±1.1[b]	89.9 ±2.8[b]
P_2	A	9.2 ±0.2[a]	92.0 ±1.1	93.0 ±0.8[ab]	90.4 ±1.9	94.6 ±2.7	93.6 ±0.9[a]	95.0 ±1.0[a]
	B	10.7 ±0.3[b]	91.8 ±0.5	92.4 ±0.4[b]	91.1 ±0.2	98.0 ±0.2	91.8 ±0.6[b]	91.2 ±1.1[b]
	C	10.5 ±0.9[b]	93.2 ±0.3	93.9 ±0.2[a]	90.3 ±2.5	96.9 ±0.8	94.7 ±0.5[a]	96.6 ±0.7[a]
P_3	A	9.0 ±1.1[a]	92.2 ±1.4	93.1 ±1.2	91.8 ±1.6	95.9 ±2.2	92.6 ±0.8	95.4 ±0.5[a]
	B	12.1 ±0.5[b]	91.8 ±0.5	92.6 ±0.4	92.0 ±0.8	98.1 ±0.7	91.8 ±0.7	89.8 ±2.0[b]
	C	13.3 ±0.3[b]	90.6 ±0.7	91.4 ±0.6	91.7 ±0.6	97.6 ±1.0	91.0 ±0.5	89.2 ±0.8[b]
P_4	A	7.8 ±0.4[a]	93.9 ±0.5[a]	94.6 ±0.4[a]	92.1 ±0.6	98.6 ±0.6	94.0 ±0.6[b]	7.6 ±0.6[a]
	B	9.1 ±0.3[b]	91.3 ±0.7[b]	92.1 ±0.6[b]	90.0 ±0.7	98.2 ±0.8	91.6 ±1.1[c]	90.0 ±2.4[b]
	C	9.1 ±0.3[b]	94.4 ±1.6[a]	95.3 ±1.3[a]	90.7 ±1.7	98.4 ±1.1	96.4 ±1.4[a]	99.4 ±3.3[a]

[1] Corrected by covariance.
[2] Reducing substances measured against lactose as reference standard.
[3] Mean ± sd. Each mean is based upon the analysis of two aliquot samples at each day collected from calves receiving the same diet. Means not sharing a common letter differ significantly between diets.(F-test).

C, respectively. Similarly, daily intakes of 15.3 and 9 g Hex. Eq. lactose per kg BW were intended in P_3 and P_4, respectively (see Appendix, page 129).

The actual daily milk intake was consistently lower than expected in animals of that age (see table 22). This forced us to reduce the allowance of diet A in P_3 to 12.2 g Hex. Eq. lactose per kg BW, instead of 15.3 g as was intended. However, even this amount proved to be too high to prevent refusals.

The results showed further that not only the animals' appetite, but also the apparent digestibility of the dietary components declined steadily in the course of this experiment. E.g. the apparent digestibility in the small intestine of dietary DM in P_1 was 92.7 ± 1.4, 93.0 ± 1.7 and 90.2 ± 1.6% in treatments A, B and C, respectively. The corresponding figures in P_5 were 90.3 ± 1.3, 88.2 ± 0.8 and 82.0 ± 0.8%, resulting in average differences between both periods of 2.4 ± 0.3, 5.2 ± 1.9 and 8.3 ± 2.4 units, respectively. Similar differences were observed for the other dietary components. Statistical analysis (F-test) indicated that the apparent digestibility of the dietary components was hardly affected by the lactose intake in P_1 and P_5 (table 22). Only the reducing substances were less efficiently digested in treatment C compared with the other treatments. The results were about equal to those observed in Exp. 9, where the apparent digestibility of reducing substances (after 3 × 12 h sampling) was 95.6% in treatment A and 92.2% in treatment C (table 20).

The constant decline in apparent digestibility was also noted in P_2, P_3 and P_4, when in each period equal amounts of Hex. Eq. were supplied. To get some insight into the effect of diet composition on apparent digestibility, covariance analysis is used to interprete the results. The results, summarized in table 22, indicate that the apparent digestibility of the dietary components in the small intestine was not substantially affected by diet composition. The differences were only slight and too small to be responsible for the significant effect of diet composition on faecal characteristics, observed in Exp. 1. The remarkable differences in Exp. 1 concerning the faecal response, when feeding different diet compositions at equal levels of lactose intake, seem to be caused by a different microbial fermentation in the lower intestine. Exp. 12 demonstrates that the level of lactose that can be tolerated in calves is an absolute amount depending on body weight, not on the percentage composition of the diet.

5.4.3. *Factors limiting lactose digestibility in the small intestine*

Increasing the intake of lactose by young calves resulted in the previous experiments in an increase of the amount digested and absorbed in the small intestine. Nevertheless, this increase did not keep pace with the increase in intake and, as a consequence, increasing amounts of carbohydrates passed through to the lower intestine (figure 14). The literature is not clear as to whether lactase activity in the brush border, or hexose absorption is the main factor causing the decline in apparent digestibility. Neither were our experimental results in treatment C informative on that point. Because of the large number of samples, the analysis of reducing substances was used as the routine method of investigating

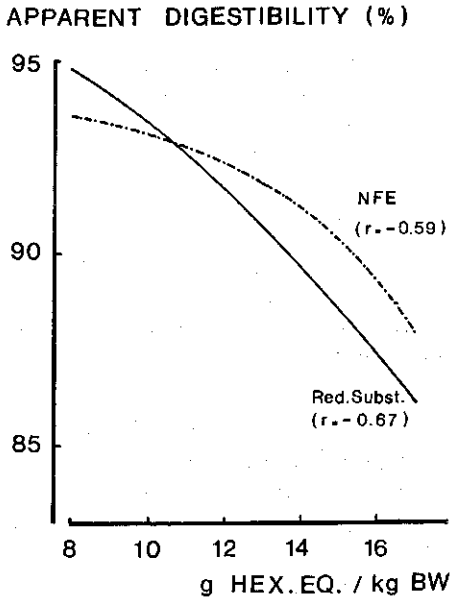

FIG. 14. The relationship between daily lactose intake and the apparent faecal digestibility of NFE and reducing substances (Exp. 12).

the dietary effect on carbohydrate flow rates in these trials. This method, however, did not differentiate between the individual carbohydrates in the samples. Neither did the NFE fraction, determined in Exp. 12 (table 22). Moreover, the results of both analyses, reducing substances and NFE, were hardly comparable, as is shown in that table. More information about the content of the individual sugars in ileal digesta was needed to get informed about the factor(s) limiting lactose digestion and absorption in calves.

With this in mind, in some of the previous experiments the glucose, galactose and lactose content were analysed in representative samples of the diets and of duodenal and ileal digesta. The reducing sugar content and individual sugars were measured according to the procedures described in section 5.2; the latter method was that of BOEHRINGER (1977). In that procedure, further referred to as method I, free glucose and galactose were determined separately in two aliquot samples after deproteinising. After about 20 minutes enzymatic digestion of the 'galactose sample' by β-galactosidase, the galactose content was measured again. The difference between the two values is assumed in this method to represent the galactose in lactose.

The analysis of reducing substances and the results by method I in milk replacer and duodenal digesta showed good agreement when glucose and galactose were corrected for their respective reducing powers in relation to the lactose reference. However, the results in ileal digesta obtained by the two analytical methods were considerably different.

In Exp. 13 we attempted to explain these differences and to estimate more accurately the sugar content in ileal digesta. In this experiment five calves, fitted with re-entrant ileal cannulae, received treatment A in two successive periods

TABLE 23. The faecal response to treatments A and C in Exp. 13.

		Treatment A	Treatment C
Number of animals		4	4
Actual daily intake (g Hex. Eq./kg BW)		8.9 ±1.3[1]	13.5 ±2.8
Faecal characteristics:			
Visual score[2]	*N*	25	9
	L	8	7
	D	–	49
pH		7.3 ±0.4[a]	5.0 ±0.9[b]

[1] Mean ± sd; means not sharing a common letter differ significantly between treatments (F-test).
[2] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.

(P_1, P_2) and treatment C in another two periods (P_3, P_4), each period lasting four days (see Appendix, page 130). Faeces were quantitatively collected in P_1 and P_3 and ileal digesta in P_2 and P_4 in each treatment. After deproteinising, each ileal and faecal sample was divided into three (duplo) aliquots. Reducing substances content was measured in one aliquot. In the second one, free glucose, free galactose and lactose were determined according to method I. The third aliquot was hydrolysed with 9.5 N HCl for 1 h and neutralized with 4 N NaOH to pH 7 and the glucose and galactose content was measured enzymatically as in method I. The latter procedure, method II, not only provided data on the total galactose content in the sample, as did also method I, but on the total glucose content as well. The results, when compared with free glucose and free galactose content analysed in the second aliquot, would provide more information on the hexose composition of the oligosaccharides in ileal digesta and faeces.

Probably because of the season, feed intake in Exp. 13 was somewhat lower than intended, ileal flow was greater and, as a result, faecal consistency less firm than in previous trials (table 23). The recovery of ileal wet digesta was approximately 28 and 76% of the oral liquid milk intake in the treatments A and C, respectively. Animals' health seemed, however, to be normal and there was no reason to suspect any disorder in the calves. Moreover, the differences in response to the treatments largely agreed with those that could be expected.

The cumulative flow of the individual carbohydrates at the distal site of the ileum, calculated from the results of the three analytical methods, are summarized in table 24. According to the results of method I, free glucose was almost negligible in ileal digesta. Small differences in glucose existed between both lactose treatments, especially from 2 to 6 h post-feeding when ileal flow rate was high. This hexose was, however, of minor importance compared with the concentration and the differences observed for the other sugars. Free galactose content in ileal digesta was higher than free glucose content. This difference was much more marked in treatment C than when control diet A was fed. According to method I, substantial amounts of lactose were collected at the distal site of the small intestine.

The results by method I suggest that the glucose absorption in milk-fed calves

TABLE 24. The cumulative flow (g/2h) of carbohydrates at the distal end of the ileum in treatments A and C (Exp. 13).

Component		Treatment	Time post-feeding (h)					
			0–2	2–4	4–6	6–8	8–10	10–12
Red. substances	(a)	A	0.67 ±0.86[1]	3.88 ± 3.92[a]	7.00 ± 5.71[a]	3.61 ±1.04[a]	1.21 ±0.86[a]	0.62 ±0.25[a]
		C	0.25 ±0.08	47.59 ±31.19[b]	51.76 ±12.30[b]	21.48 ±8.95[b]	4.94 ±0.95[b]	1.15 ±0.26[b]
Method I:[2]								
free glucose	(b)	A	0.02 ±0.03	0.04 ± 0.03	0.05 ± 0.03[a]	0.03 ±0.02	0.04 ±0.04	0.03 ±0.02
		C	0.01 ±0.00	0.46 ± 0.50	0.45 ± 0.30[b]	0.08 ±0.05	0.04 ±0.02	0.03 ±0.01
free galactose	(c)	A	0.03 ±0.06	0.34 ± 0.28[a]	0.84 ± 0.65[a]	0.42 ±0.13[a]	0.14 ±0.10[a]	0.04 ±0.03[a]
		C	0.14 ±0.13	26.64 ± 9.54[b]	18.00 ± 6.35[b]	7.01 ±3.47[b]	0.86 ±0.24[b]	0.15 ±0.04[b]
lactose	(d)	A	0.46 ±0.89	3.88 ± 3.92[a]	10.49 ±10.91[a]	4.22 ±1.77[a]	0.87 ±0.95[a]	0.15 ±0.21
		C	0.04 ±0.06	47.59 ±31.19[b]	27.76 ±13.05[b]	14.52 ±5.81[b]	6.78 ±2.09[b]	1.27 ±0.91
$\frac{a^{3}}{3.57b + 2.29c + d}$		A	1.12	0.60	0.56	0.68	0.91	1.78
		C	0.63	0.72	0.73	0.70	0.56	0.67
Method II:[2]								
total glucose		A	0.17 ±0.29	0.87 ± 1.00[a]	1.41 ± 1.06[a]	0.82 ±0.19[a]	0.30 ±0.29[a]	0.12 ±0.11
		C	0.03 ±0.01	4.21 ± 2.03[b]	4.25 ± 0.48[b]	1.84 ±0.68[b]	1.03 ±0.40[b]	0.30 ±0.12
total galactose		A	0.36 ±0.53	4.05 ± 4.83[a]	7.75 ± 7.16[a]	3.50 ±1.01[a]	0.76 ±0.51[a]	0.23 ±0.08[a]
		C	0.87 ±0.33	33.02 ±17.93[b]	35.20 ± 5.30[b]	16.23 ±6.07[b]	5.18 ±1.10[b]	1.05 ±0.58

[1] Mean ± sd; means not sharing a common letter differ significantly between treatments (t-test).
[2] Analysis by method I and II; see page 63 and 64.
[3] $\frac{\text{Reducing substances (a)}}{\text{Calculated Red. subst. from (b) + (c) + (d)}}$. The sugars are corrected for the lactose reference by 3.57 × glucose + 2.29 × galactose + lactose.

TABLE 25. The cumulative ileal flow and apparent digestibility of glucose and galactose in the small intestine (Exp. 13).

	Analytical method[1]	Treatment A		Treatment C	
		Glucose	Galactose	Glucose	Galactose
Actual daily intake (g)[2]		378.2 ±62.4[3]	378.2 ±62.4	589.6 ±98.2	589.6 ±98.2
Cumulative ileal flow (g/day)					
free		0.42 ± 0.05[a]	3.62 ± 0.22[a]	2.14 ± 0.34[b]	85.10 ±11.72[b]
as oligosaccharide	Method I	–[4]	22.88 ±24.96[a]	–	81.06 ±48.12[b]
total		–	26.50 ±25.06[a]	–	166.16 ±53.14[b]
total	Method II	7.38 ± 5.64[a]	33.30 ±22.44[a]	23.32 ± 4.04[b]	183.46 ±52.88[b]
Apparent ileal	Method I	–	93.0 ± 5.5[a]	–	71.8 ±11.0[b]
digestibility (%)	Method II	98.0 ± 1.2[a]	91.2 ± 4.8[a]	96.0 ± 1.0[b]	68.9 ±11.3[b]

[1] See page 63 and 64.
[2] The daily lactose intake provided 8.1 ± 1.0 and 11.7 ±1.7 g Hex. Eq. per kg BW in treatments A and C, respectively. The daily amounts of glucose and galactose refer to the lactose intake.
[3] Mean ± sd; means not sharing a common letter differ significantly between treatments (t-test).
[4] The amount of glucose as a component in oligosaccharides is not measured by method I. Its content is in this method presumed to be equal to 'bound' galactose.

is almost completed in the small intestine and that the galactose absorption is delayed, especially when lactose intake increases. Similar results are reported by COOMBE et al. (1971). The considerable amounts of lactose apparently recovered according to method I seem to indicate that in treatment C an insufficient digestion of lactose, as well as an incomplete absorption of galactose is responsible for the greater amount of carbohydrates flowing into the hind gut.

However, the results by method I conflicted with both, the reducing activity and those obtained by method II. The analysis on reducing substances showed that after 12 h approximately 34.0 and 254.4 g reducing substances were collected in treatments A and C, respectively. According to method I these amounts should have been 53.2 and 356.6 g, respectively. Method I thus overestimated the actual amount of reducing substances in ileal digesta by about 40–50%.

The results by method II indicated that this discrepancy was mainly caused by an overestimation of the lactose content in ileal digesta by method I (table 24, 25). The actual cumulative flow of glucose was, on average, 7.4 g per day in treatment A, consisting of 0.4 g free glucose and approximately 7 g bound in oligosaccharides. When the latter fraction would exclusively consist of lactose, only 13.2 g lactose was collected instead of 43.5 g (22.9 × 342/180), as was indicated by method I. The results by method II further showed, that the total amount of galactose in ileal digesta was underestimated by method I. The actual amount collected per day was, according to method II, approximately 33.3 g in treatment A, which was about 1.3 times as much as given by method I, indicating 3.6 g free galactose and 22.9 g bound as oligosaccharides. The digestion of the latter fraction with β-galactosidase, as is used in method I, seems to be ineffective in hydrolysing all substances in ileal digesta, containing galactose.

Similar differences were observed in treatment C (table 24, 25). The results according to method I indicated a cumulative flow of 83.2 g total glucose per day, while in method II only 23.3 g were actually measured. The former method overestimated maximal lactose content by a factor of approximately 3.8 in this treatment. The respective cumulative flows of total galactose were, according to method I and II, 166.2 and 183.5 g per day; an underestimate of about 10% compared with those actually present in treatment C, according to method II.

Based upon the results by method II, the apparent ileal digestibility of glucose declined slightly from 98.0 to 96.0%, when oral lactose intake increased (table 25). Glucose absorption seems to have been hardly limiting in treatment C. The extra amount of glucose ingested in the high lactose treatment C, 211.4 g per day, enhanced the daily absorption by approximately 195 g (92% of the additional intake). The increased lactose intake affected the galactose absorption in much greater extent. The galactose recovery in treatment C proved that only 61 g more galactose were absorbed than on control treatment A. This was about 29% of the extra amount ingested. When lactose was assumed to be represented by the glucose bound as oligosaccharide, the results by method II suggest that lactose digestion in the brush border hardly influences maximal hexose absorption in treatment C. The cumulative flow of that glucose fraction increased by 14 g per day in treatment C, which would represent 27 g lactose. Consequently the daily

TABLE 26. The apparent faecal digestibility of glucose and galactose in Exp. 13.

	Analytical method[1]	Treatment A		Treatment C	
		Glucose	Galactose	Glucose	Galactose
Actual daily intake:					
g Hex. Eq./kg BW		8.5 ±0.2[2]		13.0 ±1.2	
g Hex. Eq./day		367.1 ±42.1[3]	367.1 ±42.1[3]	676.4 ±99.7[3]	676.4 ± 99.7[3]
Faecal carbohydrate excretion (g/day)	Method I	–[4]	0.3 ± 0.4[a]	–	52.7 ± 33.6[b]
	Method II	0.4 ± 0.2[a]	0.4 ± 0.4[a]	6.8 ± 3.0[b]	57.2 ± 36.9[b]
Apparent faecal digestibility (%)	Method I	–	99.9 ± 0.1[a]	–	92.6 ± 3.6[b]
	Method II	99.9 ± 0.0[a]	99.9 ± 0.1[a]	99.0 ± 0.3[b]	91.9 ± 3.9[b]

[1] See page 63 and 64.
[2] Mean ± sd; means not sharing a common letter differ significantly between treatments (t-test).
[3] Calculated from the dietary lactose intake.
[4] See table 25.

lactose hydrolysis was 375 g higher in treatment C than in treatment A. That quantity represented approximately 93% of the extra lactose intake.

The analysis in faeces showed similar discrepancies between method I and II, as was observed in ileal digesta (table 26). Method I was less effective in the digestion of the galactose containing oligosaccharides and the lactose content in faeces was overestimated again in that analysis. The results of method II showed that lactose was almost completely digested and absorbed in treatment A. In treatment C this sugar was also completely hydrolysed, but the total glucose and galactose excretion increased to 1 and 8% of oral intake, respectively. Most of the sugars were found as free hexoses. Oligosaccharides were of minor importance in the faeces, although the fraction of 'bound' galactose increased significantly to 10% of the total amount of faecal galactose in the high lactose treatment.

The comparison of the results summarized in table 25 and 26 clearly shows that most sugars entering the colon are fermented by the microbial population in the hind gut. In treatment A about 95% of the glucose and 99% of the galactose flowing into the colon were fermented there. The corresponding figures for treatment C were 75 and 73%.

The results in Exp. 13 suggest that lactose is negligible in ileal digesta and faeces in relation to other oligosaccharides. The latter components seem to contain approximately 4.5 to 4.6 times as much galactose as glucose molecules. These compounds were responsible for the differences between the results of the separate analytical methods. Although they represent a substantial fraction of the carbohydrates in ileal digesta, their content in the diet is negligible, because no discrepancy was observed between the results of the analytical methods in milk samples, nor in duodenal digesta. It strongly suggests that the oligosaccharides are synthesized in the small intestine during lactose digestion.

The conversion of lactose into other oligosaccharides during lactose hydrolysis of whey, skim milk or lactose is described in the literature (ROBERTS et al., 1953; OLLING, 1972; WIERZBICKI et al., 1973; TOBA et al., 1978). The former authors found similar compounds in the faeces of milk-fed rats. Further analysis to differentiate between these compounds showed a large variety of carbohydrates synthesized in the digestion by lactase. TOBA et al. (1978) measured 11 to 12 different oligosaccharides when β-galactosidase was added to lactose; 5 of them proved to be disaccharides. OLLING (1972) postulated that the enzyme lactase should be considered as a galactosyl transferring enzyme. Lactose is hydrolysed into glucose and galactose when water is the acceptor of the galactosyl group. However, when galactosyl is transferred to glucose, galactose or disaccharides, e.g. lactose and galactobiose, di- or trisaccharides are synthesized. Some of these oligosaccharides consist exclusively of galactose molecules (figure 15).

The results obtained in Exp. 13 strongly suggest that lactose conversion into di- and trimers of galactose occurs in the small intestine of calves. The discrepancy between sugar analyses and the reducing substances content in the samples provides good evidence for that suggestion. ROBERTS et al. (1953) observed that

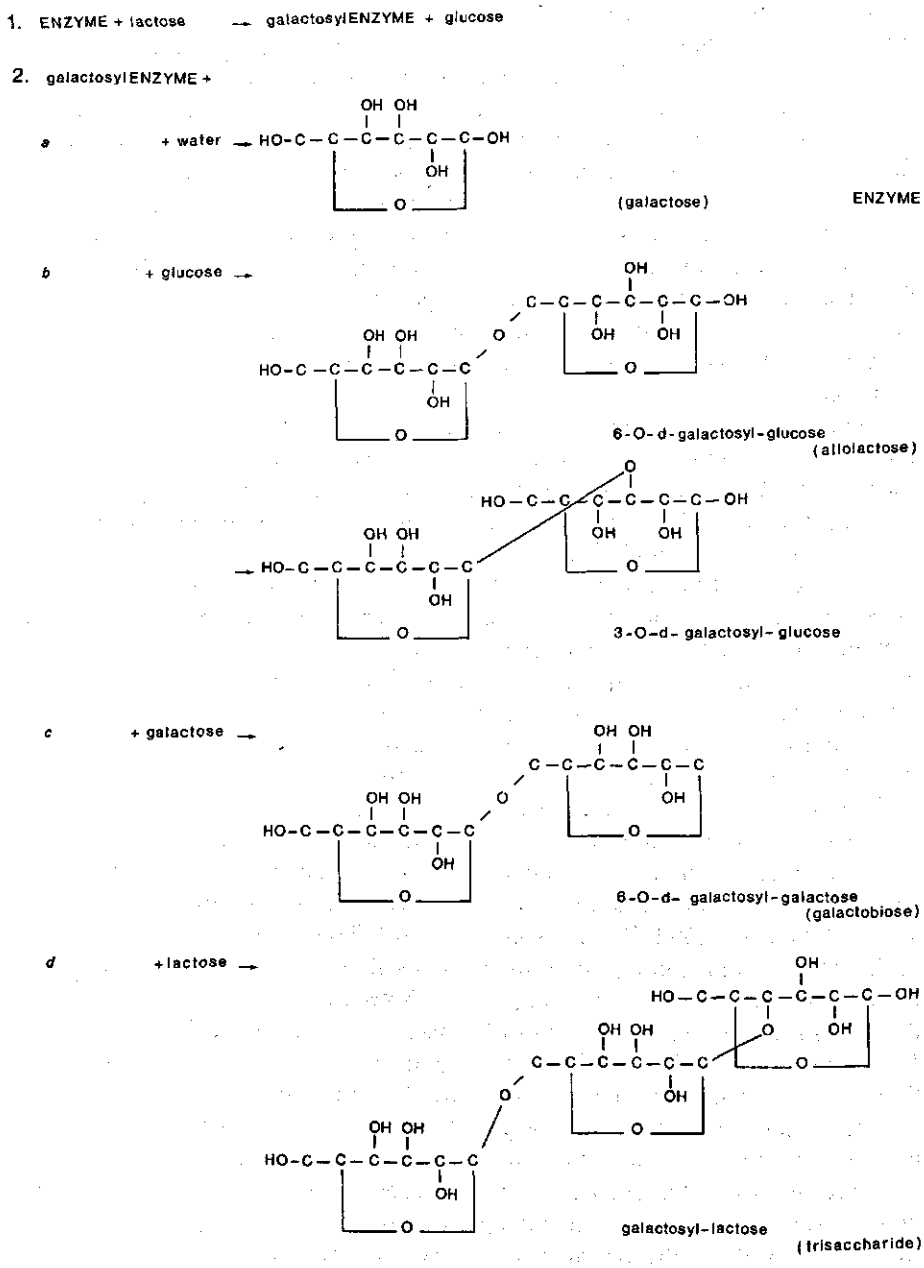

FIG. 15. Reactions involved in the transgalactosylation by lactase (OLLING, 1972).

the reducing power increases in the order: oligosaccharides < lactose < galactose < glucose. In our experiments the reducing substances content in ileal digesta was in fact lower than that expected from the free glucose, free galactose and 'lactose' content. Whether or not conversion of lactose occurs in calves was investigated in Exp. 14.

In that experiment two diets were fed to ten calves, fitted with re-entrant ileal cannulae, and to eight non-fistulated calves (see Appendix, page 131). In this experiment a high intake of lactose, treatment C, was compared with a similar intake of free hexoses, treatment C'. Both treatments allowed equal daily intakes; approximately 16–17 g Hex. Eq. per kg BW. The carbohydrates in the latter milk, diet C', consisted mainly of glucose and galactose. The composition of that diet was the same as the high lactose diet C, except that the lactose in diet C was replaced by its hexoses and the skim milk powder by another skim milk powder, containing hydrolysed lactose*). The comparison of these treatments provided the opportunity to investigate, whether or not the lactase digestion in the brush border would be important in ileal wet digesta flow rate and carbohydrate composition in ileal digesta and faeces. Moreover, the faecal response to the treatments could be related to the effects of the diets on ileal flow rate of wet digesta and digesta composition. Both diets were fed for eight days to the fistulated animals; two successive periods of four days. In the first period faeces were quantitatively collected, in the four following days the ileal digesta. Diet C was fed in P_1 and P_2, diet C' in P_3 and P_4. The non-fistulated calves received the respective treatments in P_1 and P_3 to investigate more accurately the faecal responses.

Because some questions remained at the end of P_4, it was decided to enlarge the initial design. Six calves were selected from the fistulated animals to investigate the ileal digesta parameters and faecal characteristics on control treatment A (P_5). Thereafter diet C' was fed to these calves in order to re-investigate the ileal response (P_6; see Appendix, page 131).

The effect of treatments C and C' on faecal characteristics, measured in P_1 and P_3, respectively, are summarized in table 27. The responses of the fistulated calves were not conclusive on the scouring effect. The fistulated animals seemed to tolerate rather high amounts of dietary carbohydrates. Only three calves suffered scouring when the lactose diet C was fed in P_2. The other animals tolerated a daily intake of about 14.8 g Hex. Eq. per kg BW, fed for four days. In P_3, when the hexose diet C' was fed, only one animal suffered scouring. It suggested that diet was less conducive to diarrhoea. However, the daily intake was somewhat lower than in P_2 and tended to decrease further when the treatment lasted longer. Moreover, almost all animals suffered severe diarrhoea during the following four days (P_4), when ileal digesta was collected in the same treatment. According to the standard procedure in our experiments, ileal digesta was quantitatively collected during 12 h per day and replaced by diluted, *Normal*

*) Lactalac M®; C.C.F., Leeuwarden. Approximately 96% of the lactose was hydrolysed into glucose and galactose. When analysed, lysine proved to be 96% available. The protein quality in both milk replacers was therefore not different.

TABLE 27. The effect of treatments C and C′ on faecal characteristics in Exp. 14.

		Treatment C (lactose diet)	Treatment C′ (hexose diet)
Fistulated calves.			
Number of animals		9	9
Actual daily intake (g Hex.Eq./kg BW)		14.8 ±1.0[a] [1]	12.0 ±2.3[b]
Faecal characteristics:			
Visual score[2]	*N*	31	26
	L	12	4
	D	18	6
pH		5.8 ±1.0[a]	5.7 ±0.9[b]
Non-fistulated calves.			
Number of animals		8	8
Actual daily intake (g Hex.Eq./kg BW)		15.6 ±1.3	14.8 ±2.0
Faecal characteristics:			
Visual score	*N*	19	22
	L	35	20
	D	23	23
pH		5.4 ±0.9[a]	6.0 ±1.0[b]

[1] Mean ± sd; means not sharing a common letter differ significantly between treatments (t-test).
[2] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.

faeces. This procedure had always prevented scouring in the previous experiments (and also in P_2), irrespective of the diet fed. The scouring response observed in P_4 suggested that in P_3 the diarrhoeic effect of treatment C′ was not very evident in the fistulated calves. The response to treatment C′ in the non-fistulated animals confirmed that view. Most of these animals responded to both diets, C and C′, with scouring (table 27). The response to diet C was about similar to that observed in previous trials. Diet C′ seemed to have about equal diarrhoeic property as the high lactose diet, when fed for four days.

However, the scouring effect of diet C′ in P_4 when collecting ileal digesta, and its effect on the general health of the calves suggested that the hexose treatment was more risky in these animals than an equal intake of lactose. Scouring persisted when treatment C′ was stopped and the animals were changed to control diet A. Calves' condition was notably poor, especially in the fistulated group, and feed intake remained substantially lower than normal. Moreover, some animals had remarkable high amounts of erythrocytes in ileal digesta. That may have been an indication of lesions occurring in the intestinal mucosa when feeding the hexose diet C′. In the literature similar disorders are cited as occurring after massive doses of glucose (55, 56, 94, 148, 193). Their weak condition forced us to remove four calves from the experiment during and after P_4.

Diet C′ enhanced the flow rate of ileal wet digesta more than diet C (figure 16). The maximal flow rate was reached slightly earlier as well, suggesting that diet C′ had reduced digesta transit time as far as the end of the ileum. The pattern of the sugar flow rate curves reflected that of wet digesta. It suggests that the increased

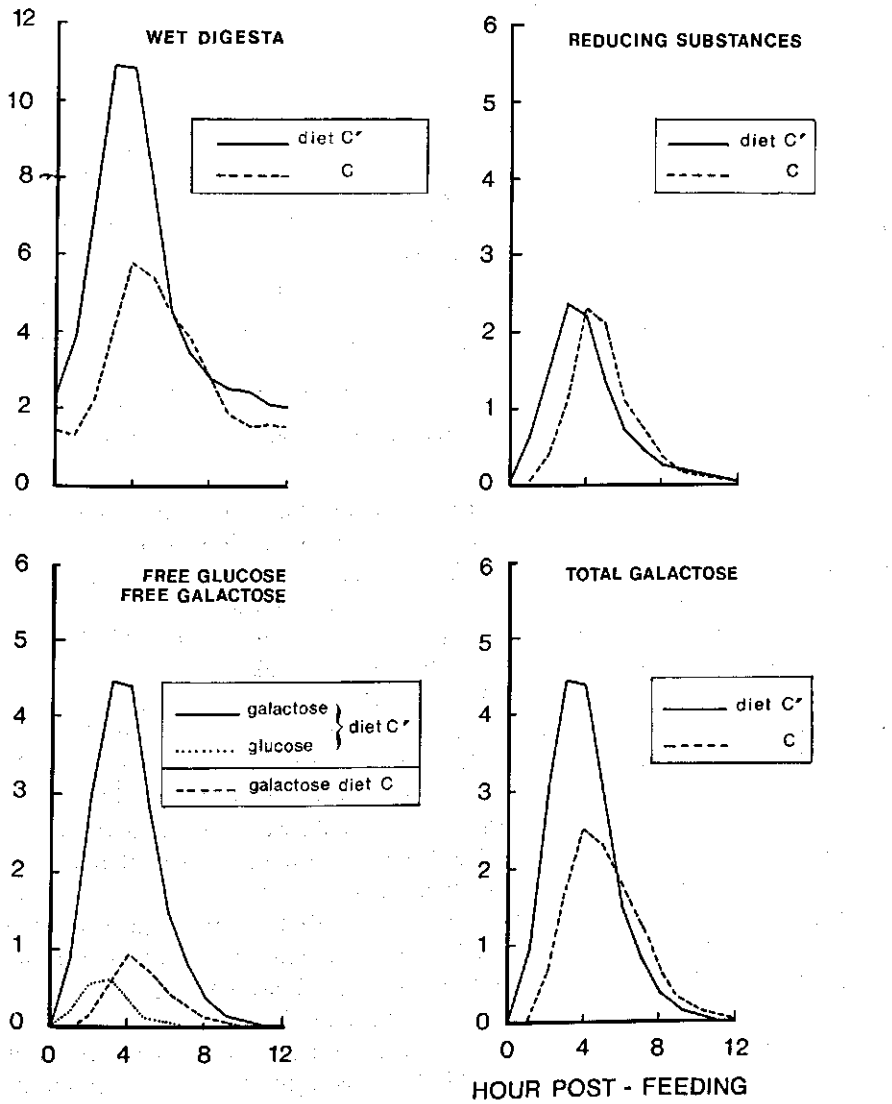

FIG. 16. The average flow rates of ileal wet digesta, reducing substances, glucose and galactose, feeding diet C and C' to calves (Exp. 15). The hexoses were analysed according to method I and II.

carbohydrate flow rates are largely responsible for the higher weights of wet digesta. The ileal recovery of wet digesta in the lactose treatment C was approximately 36% of the oral liquid milk intake (table 28). This quantity was not substantially different from that usually observed in this treatment. In P_4, feeding diet C', the average recovery was slightly higher than in treatment C.

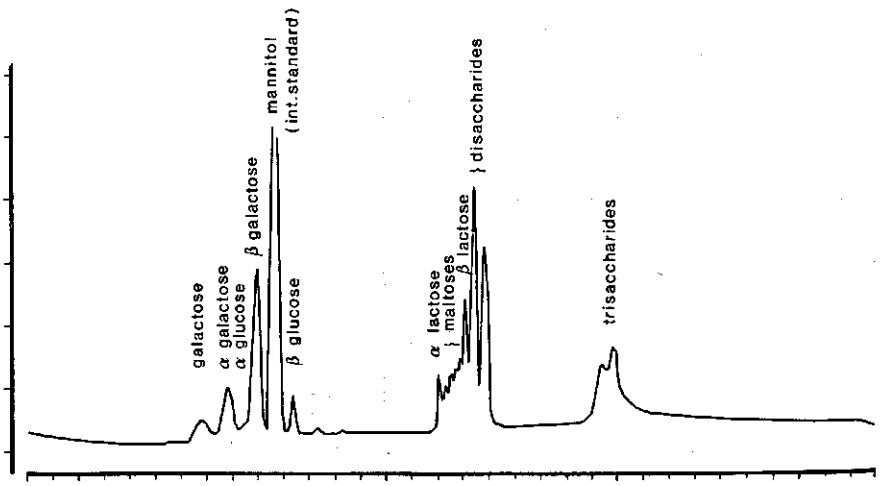

FIG. 17. Gas chromatogram of trimethylsilylether derivatives of sugars in ileal digesta after feeding diet C (Exp. 15).

However, the difference between both treatments was small compared with those between the responses of the individual calves on the hexose diet. In some animals ileal digesta flow was hardly affected and the recovery of wet digesta was almost equal to that generally observed in treatment A (about 20 % of liquid milk intake). In other calves, the recovery of wet digesta was considerably higher, occasionally exceeding 100 % of the oral liquid milk intake.

The recovery of wet digesta in treatment A (P_5), 38 % of the oral liquid milk intake, was higher than expected and about as high as in P_2, feeding the high lactose diet C. The high recovery was the more remarkable, because the animals received control diet A for about two weeks before sampling started. The calves seemed still to be suffering from a carry-over effect of treatment C'. The results in P_6 confirmed that supposition. Ileal wet digesta recovery was in that period substantially higher than for the same treatment in P_4.

Each ileal and faecal sample was analysed for carbohydrate content, according to method I. Aliquot samples of each treatment were analysed for the composition of the oligosaccharide fraction, according to OLLING (1972) *). Both methods of analysis agreed in the average free glucose and free galactose content, proving the aliquot samples to be representative for the mean effects of the dietary treatments and showing that both methods of analysis were comparable in respect of the free hexose content in the samples. The results of the latter analysis are summarized in table 28. They clearly show that the carbohydrate fraction in ileal digesta does not consist only of lactose or its hexoses, but mainly of other oligosaccharides, when lactose is the main dietary carbohydrate (figure 17). Free glucose recovery at the distal end of the ileum was almost negligible in the high lactose treatment (0.1 % of glucose intake). Total glucose (about 2 % of

*) These analyses were carried out by the C.C.F., Leeuwarden.

TABLE 28. The recovery and apparent digestibility of individual sugars in the small intestine, when feeding diets containing either lactose, diet A and C, or glucose and galactose, diet C′ (Exp. 14).

	P_2 Diet C	P_4 Diet C′	P_5 Diet A	P_6 Diet C′
Number of animals	9	6	6	6
Actual daily intake (g Hex.Eq./kg BW)	14.4 ± 1.3[1]	14.0 ± 1.5	8.0 ± 0.2	13.5 ± 0.6
Recovery of wet digesta (% of liquid milk intake)	36.2 ±10.8	48.2 ±38.0	38.2 ±15.4	63.2 ±30.5
Recovery of individual sugars (Hex. Eq. in % of oral hexose)[2]:				
glucose	0.14	1.88	0.10	0.42
galactose	2.58	19.03	2.74	17.46
lactose	0.91	0.10	1.06	0.10
maltose	0.91	–	2.13	0.56
disaccharides	3.63	1.40	4.19	1.33
trisaccharides	6.15	1.19	3.20	0.07
Apparent digestibility (%):				
total glucose[3]	98.0	98.0	96.7	98.9
total galactose[4]	86.7	78.3	88.8	81.0
lactose	99.1	98.2	98.9	97.8
total hexoses	92.4	87.9	92.7	89.6

[1] Mean ± sd.
[2] Analysed according to OLLING (1972). Glucose and maltose are expressed in % of glucose intake; galactose, di- and trisaccharides in % of galactose intake; lactose in % of total Hex. Eq. intake.
[3] Calculated from glucose, maltose and lactose.
[4] Calculated from galactose, lactose, di- and trisaccharides.

oral intake) did not importantly contribute to the carbohydrates in ileal digesta in this treatment. The recovery of free galactose was 2.6% of oral galactose intake. The remaining galactose fraction was either bound in lactose (0.9% of oral intake), in other disaccharides (3.6% of oral intake, when these are presumed to represent dimers of galactose), or in trisaccharides (6.2% of oral intake, when these are assumed to be trioses of galactose). These results confirm that lactose is almost completely digested by lactase in the small intestine, even when large amounts lactose are fed. They also confirm that conversion of galactosyl molecules to other hexoses or oligosaccharides occurs during the digestion in the small intestine of calves.

The apparent ileal digestibility of galactose was 86.7% in P_2. That was approximately 18 units higher than that observed in the same treatment in Exp. 13 (table 25). It suggests an efficient digestion of the carbohydrates in these calves, which might have been the reason for the relatively mild faecal responses observed in these fistulated animals in P_1 and P_3.

The free glucose recovery at the end of the ileum was slightly higher in P_4, treatment C′, than in P_2 on diet C (table 28). The total amount of glucose recovered was, however, approximately the same in both treatments. Free galactose recovery, approximately 19% of oral galactose intake, was increased con-

siderably on the hexose diet compared with the lactose diet. However, when feeding hexose, only 2.7 % of oral galactose was bound in oligosaccharides. The minor importance of the oligosaccharides in this treatment agreed with the view that in ileal digesta these compounds mainly originate from the conversion of lactose by lactase. The apparent ileal digestibility of galactose (78.3 %) was lower in this treatment than in treatment C. This difference may have reflected the kinetic advantage in absorption of the hexoses of lactose digested in the brush border, as is generally reported in the literature. However, the intestinal disorders observed during and after treatment C′, may also have affected the absorption of the hexoses.

The results in P_5 when calves received control diet A confirmed the presence of several oligosaccharides in ileal digesta when feeding lactose (table 28). The apparent carbohydrate digestibility in the small intestine was, however, slightly lower in this period than would have been expected from the results in P_2 and in Exp. 13 (table 25). Total galactose recovery was 11.2 % in P_5; 2.7 % was recovered as free galactose, 1.0 % as lactose, 4.2 % bound in other disaccharides and 3.2 % in trioses. Total glucose recovery, 3.3 % of oral glucose intake, was lower than the total galactose recovery. Only 0.1 % was recovered as free glucose; the rest was bound in oligosaccharides, e.g. maltose.

The carbohydrate recovery in P_6, when treatment C′ was repeated, confirmed almost exactly that obtained in P_4. Approximately 1.1 % glucose was recovered in the ileal digesta; half of that as free glucose. Free galactose recovery was again considerably higher than in the two preceding lactose treatments. Its recovery was approximately 17.5 % of oral galactose intake and represented almost all the galactose recovered (19.0 % of oral intake). The carbohydrate recovery in P_6 was almost equal to that in P_3, but total wet digesta recovery increased by 15 units

TABLE 29. The effect of the replacement of high lactose intake (treatment C) by high intakes of glucose and galactose (treatment C′) on the apparent faecal digestibility (Exp. 14).

	Treatment C	Treatment C′
Number of animals	9	9
Actual daily intake (g Hex. Eq./kg BW)	14.8 ±1.0[a] [1]	12.5 ± 1.9[b]
Apparent faecal digestibility (%):		
DM	95.2 ±1.8	96.2 ± 1.7
OM	95.6 ±1.7[a]	97.8 ± 1.5[b]
N	88.9 ±3.8	91.8 ± 3.4
EE	90.7 ±5.9	81.1 ±18.2
NFE	97.8 ±1.8[a]	99.0 ± 0.8[b]
Total glucose[2]	99.7	100.–
Total galactose[2]	99.5	100.–
Total hexoses	99.6	100.–

[1] Mean ± sd; means not sharing a common letter differ significantly between treatments (t-test).
[2] See table 28.

(table 28), indicating a higher water flow at the distal end of the ileum in this period, not directly related to the content of soluble carbohydrates. This was quite different from the experience in the lactose treatments so far. It presumably indicates that in this period other disturbances in the digestive process occurred, probably because of the adverse effect of diet C′ in P_3 and P_4.

Faecal samples, collected in individual animals in P_1 and P_3, were analysed to investigate the apparent total digestibility of the main dietary components in diets C and C′. The results, given in table 29, indicate that the diets hardly differed in apparent faecal digestibility. Crude fat was substantially less efficiently digested in treatment C′. That lower digestibility, however, referred only to three calves, showing a remarkable low apparent fat digestibility; 76.7, 63.9 and 44.8 %. It was not clear, whether or not the intestinal disorders described had interfered with the apparent digestibility of dietary fat.

As with ileal samples, aliquot faecal samples were analysed for each treatment on oligosaccharide composition. Free glucose and free galactose were the main carbohydrates excreted in the faeces. Traces of lactose, maltose and other disaccharides, e.g. galactobiose, were found in faeces in treatment C but no trisaccharides. The total recovery of glucose and galactose in the faeces, although almost negligible in both treatments, was slightly higher when lactose was fed.

The results in Exp. 13 and 14 indicate that conversion of lactose into other oligosaccharides occurs in the small intestine of calves. However, these components did not seem to inhibit the galactose absorption. The apparent digestibility of that hexose was slightly higher when lactose was fed instead of the individual hexoses, glucose and galactose. It is not clear from these experiments, whether or not the di- and trisaccharides affect the scouring property of the diet.

Lactase is not limiting lactose absorption. Lactose recovery at the end of the small intestine was about 1 % of oral intake in the lactose treatment. This amount was of minor importance in relation to the galactose recovery, ca. 12–13 % of oral intake, and may have been, to some extent at least, a result of transgalactosylation. It indicates that the lactase hydrolysis was sufficiently efficient in these experiments. The limits put on galactose absorption seem to be mainly responsible for the changes in ileal wet digesta flow rate and composition when high amounts of dietary lactose, or its hexoses, are fed to young calves.

5.5. The diarrhoeic effect of carbohydrates in colonic digesta

Our results so far show a higher wet digesta and carbohydrate flow into the lower intestine when scour-inducing levels of dietary lactose were given to milk-fed calves. It is generally accepted in the literature (Chpt. 2) that in these circumstances scouring is the result of microbial fermentation of the carbohydrates in the colonic lumen. Our experiments provide evidence that such an effect occurs. However, the results in Exp. 5 (Chpt. 3), in which the scouring effect of lactose infusions in colonic digesta was measured, were not completely

convincing in this respect. Even the infusate of 20 % of oral NFE intake resulted in a milder scouring response than was usually observed in treatment C. The validity of this observation is open to question because it was made on only two calves, so a comparison with results of treatment C may not be justified. However, it may also be possible that the change in carbohydrate composition in colonic digesta is not the only factor responsible for the scouring phenomenon. The effect of high lactose intake on the absorption of other dietary components, e.g. minerals, may also be important in this respect.

Therefore, the diarrhoeic effect of ileal digesta, collected in scouring calves, and that of the carbohydrate content in colonic digesta were investigated in two experiments, Exp. 11 and 8, respectively. In Exp. 11 (P_5, P_6) the diarrhoeic effect of ileal wet digesta, collected in calves receiving treatment D was tested (see Appendix, page 127). That treatment, supplying 8 g Hex. Eq. lactose and 3 g Hex. Eq. sucrose per day per kg BW, was chosen specifically for this purpose because its scouring effect in calves was well defined. The diet was fed to all calves in P_5 to investigate the individual ileal and faecal responses of the animals. One calf, suffering a mild lung infection in that experimental period, was not included in the results, summarized in table 21 and 30, respectively. Afterwards, in P_6, the animals were allotted to two groups; one of those received diet D and the other one control diet A. The ileal digesta collected in the calves receiving diet D were quantitatively infused into one of the calves receiving diet A, and vice versa (see Appendix, page 127).

In P_5, treatment D quickly resulted in high ileal flow rates of wet digesta and scouring in all animals. The 'crossing' of ileal digesta in P_6 induced comparable

TABLE 30. The scouring effect of treatment D alone and when the ileal digesta were infused into the hind gut of animals receiving treatment A or vice versa.

		P_5	P_6	
		Treatment D	Treatment A	Treatment D
Number of animals		5	3	3
Actual daily intake (g Hex. Eq./kg BW):				
lactose		7.0 ±0.7[1]	7.1 ±0.6	7.6 ± 0.1
sucrose		2.6 ±0.2		2.9 ± 0.0
Ileal recovery of wet digesta (% of liquid milk intake)			10.6 ±2.2[a]	69.6 ±10.9[b]
Faecal characteristics:				
Visual score[2]	*N*	3	4[3]	2[3]
	L	2	7	0
	D	17	18	0
pH			5.7 ±0.7[a]	7.6 ± 0.4[b]

[1] Mean ± sd; means not sharing a common letter differ significantly between treatments (t-test).
[2] Number of samples; *N* is *Normal*, *L* is *Loose* and *D* is *Diarrhoea*.
[3] The ileal digesta collected in P_6 were infused into the distal ileal cannulae of counterpart calves, receiving the other diet.

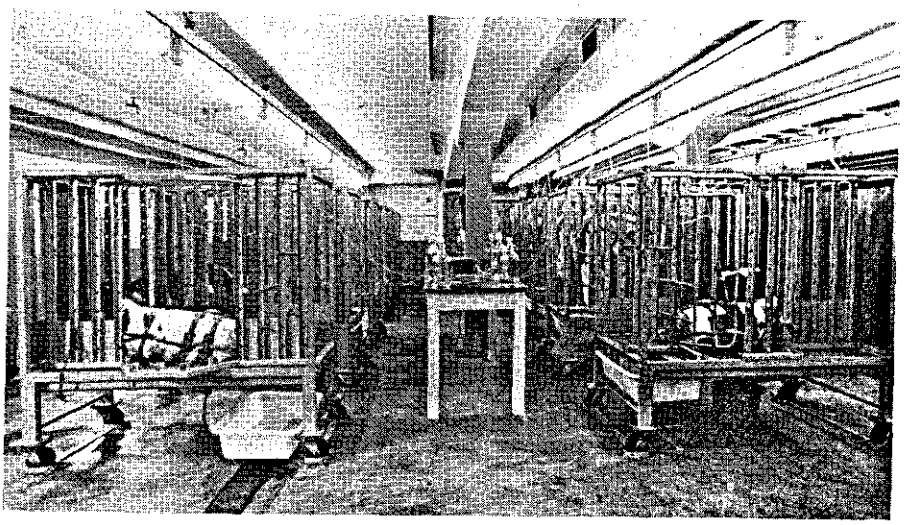

FIG. 18. Infusion of carbohydrates into the lower intestine (Exp. 8).

disturbances in the animals ingesting control diet A. Feed intake in that group fell to approximately 70% of the intended level of milk intake (table 30). Conversely, the animals ingesting diet D did not respond to the dietary treatment, neither with scouring, nor with feed intake. The small number of faecal samples collected in these animals were *Normal*. These results proved that the factors responsible for dietary scouring were already present in the ileal digesta.

The role of the carbohydrate content in ileal digesta in fermentative scours was investigated in Exp. 8. Twelve ileal fistulated calves were allotted at random to three groups and received control diet A (see Appendix, page 124). Carbohydrate infusions were introduced into the lower intestine at levels of 40% of the oral intake. This level was based on the results in previous experiments in which treatment C had produced severe scouring in the most susceptible calves. In these animals about 40% of the oral intake of reducing substances was recovered at the distal end of the ileum. In two experimental periods (P_1, P_3) lactose at 40%, and galactose at 17.5 and 40% of the NFE intake were infused continuously into the ileal cannulae. The 17.5% level of galactose was equal to 40% of oral reducing substances intake. All sugars were infused in aqueous solution, isotonic with ileal digesta (312 m osmol/L). An isotonic NaCl infusion served as control treatment in this experiment and was tested in P_2.

The results of Exp. 8 are summarized in table 31. The introduction of the carbohydrates in colonic lumen quickly reduced faecal consistency, pH and DM content. The scouring response, in particular to the 40% carbohydrate infusates, was more serious than generally observed in treatment C, but very similar to that in the most susceptible calves. The effect of these infusates led to the conclusion that the high carbohydrate flow into the lower intestine was primarily responsible for the disturbances arising from dietary

TABLE 31. The effect of sugar infusions into the lower intestine on faecal characteristics (Exp. 8).

	Ileal infusion in % of oral NFE intake[1]			
	Control (NaCl)	Galactose 17.5%[2]	Galactose 40%	Lactose 40%
Number of animals	8	8	8	8
Actual daily intake (g Hex. Eq./kg BW)	8.4 ±0.2[3]	8.6 ± 0.1	8.6 ± 0.2	8.6 ±0.1
Actual amounts infused:				
g Hex. Eq./kg BW	–	1.5 ± 0.6	3.4 ± 0.2	3.3 ±0.4
% of oral NFE intake	–	17.3 ± 0.6	39.2 ± 2.0	39.0 ±4.6
Faecal characteristics:				
Visual score[4] *N*	33	12	5	5
L	3	14	1	9
D	1	8	39	25
pH	7.4 ±0.4[a]	5.9 ± 1.1[b]	5.0 ± 1.0[c]	4.6 ±1.1[c]
DM (%)	13.0 ±0.1[a]	9.0 ± 2.0[b]	4.8 ± 0.1[d]	7.9 ±0.5[e]
NFE (% in faecal DM)	19.2 ±0.8[a]	36.4 ±14.9[b]	73.3 ±10.7[d]	64.4 ±7.9[e]

[1] The components were infused in an aqueous solution, isotonic to ileal digesta (312 m osmol/L).
[2] Equal to 40% of oral reducing substances.
[3] Mean ± sd; means not sharing a common letter differ significantly between treatments (F-test).
[4] Number of samples; *N* is *Normal*, *L* is *Loose* and *D* is *Diarrhoea*.

lactose as observed in the previous experiments.

Some interesting, significant differences existed between the results on the two 40% infusates. The galactose treatment was slightly more prone to produce scouring than was the lactose infusion. The lower DM content, higher faecal pH and higher NFE content in the DM seem, however, to indicate that less galactose than lactose is fermented in the hind gut. That result might suggest that the scouring phenomenon was not caused only by the end products of the microbial carbohydrate fermentation; the carbohydrates themselves seemed to exert a direct effect. It seems likely that the 40% galactose treatment, having a higher osmotic activity than lactose, reduced digesta transit time in the hind gut. If so, less time would be left for the microbes to ferment the infusate, which would explain the higher pH and NFE content in this treatment. Transit time was not measured in this experiment. Further research would be needed to determine the significance of transit time in the hind gut in fermentative scouring.

The effect of the 17.5% galactose infusion was less marked than that of lactose in equal amounts of reducing substances. This was to be expected having regard to the chemical composition of ileal digesta observed on treatment C in Exp. 13 and 14 (section 5.4.3).

The results in Exp. 8 and Exp. 11 show that the changes in ileal digesta, when feeding an excess of lactose to calves, are responsible for the scouring phenomenon. They also suggest that the osmotic effects, either from the carbohydrate compounds, or from their end products after microbial fermentation, are pri-

marily responsible for the enhanced water excretion in the faeces. It seems likely that in such circumstances the transit time of digesta in the large intestine is also affected.

5.6. The effect of dietary lactose on water excretion and mineral absorption and retention

The considerable loss of water with faeces in the scouring animals may have changed their total water excretion and mineral absorption and retention. One of the objectives in our work was to get more information about these effects, which may be even more important for animal health than the lactose induced diarrhoea itself.

In several trials it was intended to collect urine and faeces separately so as to investigate the effect of the dietary carbohydrate treatments on water excretion. However, quantitative collection of faeces and urine separately proved to be rather difficult in the scouring calves. The watery faeces easily leaked from the collection bags into the urine. Many samples could not be used because of contamination with faeces. Reliable information was only obtained in Exp. 9, 14 and 15. The results obtained in these trials reflect the change in urine and faecal water excretion; they do not give an exact measurement of the water balance in these calves. It was assumed that neither the evaporation of water via the lungs and skin, nor the metabolic water production was substantially changed by the high intakes of dietary lactose. It was further assumed that the total urine excretion was representative of the water excretion by that path.

In Exp. 9 urine and faecal water excretion were quantitatively measured in the non-fistulated 'control' group, receiving treatment A or C (see Appendix, page 125). In treatment A all 38 faecal samples were judged to be *Normal* (table 18).

Table 32. The effect of high lactose intake on the excretion of water with faeces and urine (Exp. 9).

	Treatment A		Treatment C	
Number of animals	4		4	
Actual daily intake (g Hex. Eq./kg BW)	9.6 ±0.6[1]		15.8 ±1.4	
Faecal pH	8.0 ±0.4[a]		6.6 ±1.0[b]	
Faecal DM (%)	14.5 ±2.4[a]		11.9 ±3.9[b]	
	g/day	%	g/day	%
Daily water intake	7256 ±220	100	7289 ± 16	100
Daily water excretion:				
Faeces	175 ± 27[a]	2.4 ±0.3[a]	586 ± 92[b]	8.0 ±1.2[b]
Urine	3905 ±406[a]	53.8 ±4.0[a]	2895 ±375[b]	39.7 ±5.2[b]
Total	4080 ±433	56.2 ±4.3	3481 ±282	47.8 ±4.0

[1] Mean ± sd; means not sharing a common letter differ significantly between treatments (t-test).

Scouring frequency and the total weight of faeces increased considerably in treatment C. On that treatment 44 samples were scored as *Normal*, 24 as *Loose* and 18 as *Diarrhoea*. Faecal water excretion increased from 2.4 to 8.0% of oral water intake when the calves received the scouring diet (table 32). The decline in urine excretion, however, more than compensated for that difference. The total excretion of urine plus faecal water was not substantially different in the treatments A and C. Similar results were obtained in Exp. 14 and Exp. 15.

The changes in the pathway of water excretion were not only observed when the daily intake of lactose was increased, but also within the high carbohydrate treatments. That is e.g. illustrated by the results in Exp. 14 on diets C and C' (see Appendix, page 131). The fistulated calves, that did not scour or experienced only mildly scouring, excreted a small proportion of water in their faeces. That proportion increased markedly in calves, in which at least 50% of the faecal samples were scored as *Loose* or *Diarrhoea*. It was balanced by an equal reduction in urine excretion; the total water excretion did not change substantially. The former, less susceptible group of calves excreted approximately 4% of their urine + faecal water via the faeces. That proportion increased to 20% in the other group, being more susceptible to the scouring property of the diets. Regression of the faecal water excretion, expressed as proportion of the total water excretion, against faecal pH ($r = -0.93$) indicated that the proportion of faecal water increased by 13 units, when faecal pH decreased 1 unit.

These results suggest that lactose induced scouring in milk-fed calves does not necessarily change the water balance and result in dehydration, as in calves suffering scours of pathogenic origin (e. g. ROY, 1969; FISHER et al., 1971; DALLENGA, 1976). Only the pathway of water excretion changed in our trials.

In agreement with general views, sugar content of the urine increased with the intake of lactose. In table 33 the results of sugar analyses in aliquot urine samples are summarized. The sugar excretion in the urine was low in treatment A; approximately 0.04 and 0.05% of the ingested glucose and galactose, respectively, were recovered in the urine. Treatment B slightly reduced urine excretion, but increased urinary glucose and, in particular, urinary galactose excretion. In

TABLE 33. The effect of dietary lactose on the excretion of glucose and galactose in the urine[1] (Exp. 15).

	Treatment A	Treatment B	Treatment C
Number of animals	8	8	7
Actual daily intake (g Hex. Eq./kg BW)	10.4 ±0.1	14.0 ± 0.0	16.8 ±0.7
Daily urinary excretion:			
urine (% of water intake)	59.1 ±6.5[a]	51.1 ±12.2[a]	28.1 ±8.8[b]
glucose (% of glucose intake)	0.04	0.12	1.10
galactose (% of galactose intake)	0.05	1.34	1.44

[1] Glucose and galactose content were analysed by method II (see page 64) in aliquot urine samples per treatment.

[2] Mean ± sd; means not sharing a common letter differ significantly between treatments (F-test).

treatment C urine excretion was significantly reduced. However, the glucose and galactose recovery in the urine were further increased to 1.10 and 1.44 % of oral glucose and galactose intake, respectively, which was somewhat higher than that excreted in the faeces, 0.3 and 0.5 %, respectively (table 29).

The effect of the dietary treatments A, B and C on the absorption and excretion of macro minerals in milk-fed calves was investigated in Exp. 15. These diets were fed to twenty calves, ten of them fitted with re-entrant ileal cannulae, in three subsequent experimental periods of five days (see Appendix, page 133). The absorption of Na, K, Cl, Ca, P and Mg in the small intestine was investigated in the fistulated calves. The non-fistulated animals served to measure the apparent faecal absorption and the retention of these elements. Faeces and urine were quantitatively collected in these animals for that purpose. More detailed information on the design is given in the Appendix, page 133.

Treatments B and C quickly resulted in scouring in the animals used in this experiment (table 35). In treatment C scouring was so serious that we were forced to stop the treatment after three days instead of the five days intended. The fistulated calves on treatments B and C showed high ileal flow rates of wet digesta (figure 19). This is consistent with the scouring observed on these treatments in the non-fistulated group. It is probable, therefore, that this comparability could be extended to the mineral portion of the digesta and that the results measured in the one group of animals are transferable to the other.

The apparent absorption of Ca, P and Mg in the small intestine confirmed the relatively high availability of these macro elements in milk-fed calves (table 34). Similar results were measured in Exp. 3 and 5 (Chpt. 3) and cited in the literature (e.g. SMITH, 1962). The apparent absorption of Ca was slightly, but significantly reduced in the high lactose treatments. The effect on the apparent absorption of

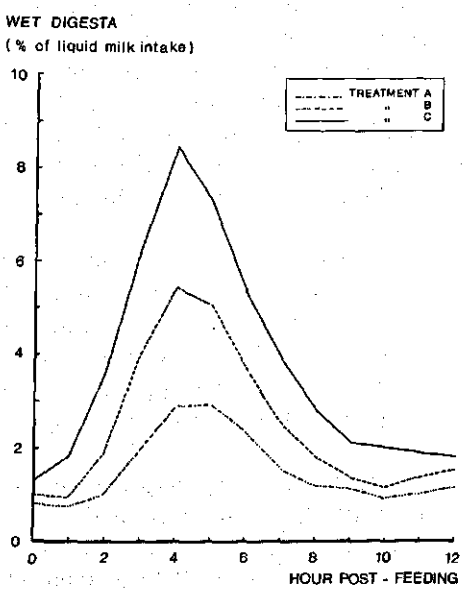

FIG. 19. The effect of the treatments A, B and C on ileal flow rate of wet digesta (Exp. 16).

TABLE 34. The effect of lactose intake on the apparent absorption of macro elements in the small intestine (Exp. 15).

	Treatment A	Treatment B	Treatment C
Number of animals	9	9	9
Actual daily intake (g Hex. Eq./kg BW)	10.0 ± 0.8[a1]	12.4 ± 1.3[a]	13.9 ± 2.4[b]
Recovery of ileal wet digesta (% of oral liquid milk intake)	18.9 ± 3.2[a]	30.8 ± 6.4[b]	60.8 ± 18.5[c]
Mineral apparent absorption in the small intestine (% of oral intake):			
Na	47.7 ± 15.4[a]	25.6 ± 15.1[ab]	6.3 ± 37.5[b]
K	95.1 ± 1.5[a]	90.2 ± 3.1[b]	87.0 ± 4.2[c]
Cl	88.1 ± 2.8[a]	84.6 ± 4.4[a]	73.3 ± 11.8[b]
Ca	82.6 ± 4.0[a]	79.8 ± 4.2[a]	72.9 ± 4.4[b]
P	95.0 ± 1.7[b]	96.4 ± 0.9[a]	95.2 ± 1.6[ab]
Mg	37.2 ± 9.2	39.2 ± 5.9	38.0 ± 7.3

[1] Means ± sd; means not sharing a common letter differ significantly between treatments (F-test).

P and Mg was negligible. Treatment C significantly reduced the apparent absorption coefficient of Na, K and Cl in the small intestine. The higher coefficient of variation in that treatment reflected the large differences in response between the individual calves. The higher intakes of lactose affected in particular the apparent absorption of Na, which decreased by approximately 22 units in treatment B and 41 units in treatment C by comparison with control treatment A. Three animals even showed a negative Na absorption coefficient on the highest lactose treatment; a value of −44 % was observed in one of these calves. Cl and K absorption seemed to be less affected by dietary lactose.

The apparent faecal absorption of Ca was high in control treatment A and that of P, Na and K was almost complete (table 35). In particular Ca and P absorption were higher than in adult animals, but normal in these young animals receiving a milk substitute diet. When these data were compared with those observed at the end of the small intestine (table34), the absorption of K, Ca, P and Mg was almost completed in the small intestine. In agreement with the results in Exp. 3 and 5, approximately 50 % of the Na was absorbed in the hind gut.

The effect of high lactose intake on the apparent faecal absorption and retention of the minerals was less pronounced than observed at the distal end of the small intestine. The apparent faecal absorption of Ca was slightly and that of Na and K significantly reduced in treatments B and C. These effects were also reflected in the retention of these electrolytes. Neither the apparent faecal absorption, nor the retention of Cl, P and Mg were substantially affected by the high lactose diets.

An accurate estimation of the quantitative effects of dietary lactose on the Na and K absorption and retention was somewhat difficult because of the large differences between individual calf responses. Regression analysis proved to be rather inaccurate. The correlation coefficients ranged from 0.35 to 0.66, al-

TABLE 35. The effect of lactose intake on faecal characteristics and the apparent faecal absorption and the retention of macro elements (Exp. 15).

		Treatment A		Treatment B		Treatment C [1]	
Number of animals		8		8		7	
Actual daily intake (g Hex. Eq./kg BW)		10.4 ±0.1[a2]		14.0 ±0.0[b]		16.8 ±0.7[c]	
Faecal characteristics:							
Visual score[3]	*N*	61		24		12	
	D	4		30		35	
	L	–		54		89	
pH		6.9 ±0.6[a]		5.1 ±1.0[b]		4.5 ±0.6[c]	
		Absorption[4]	Retention[5]	Absorption	Retention	Absorption	Retention
Na		98.7 ± 0.1[a]	35.9 ±10.8[a]	93.6 ±6.5[a]	11.4 ±7.7[b]	83.3 ±13.8[b]	12.1 ±16.5[b]
K		97.9 ± 1.4[a]	26.4 ± 6.0[a]	96.4 ±2.5[a]	14.5 ±3.6[b]	88.4 ± 8.6[b]	15.4 ± 4.1[b]
Cl		98.7 ± 0.5	14.0 ± 6.9	98.8 ±0.4	16.5 ±3.6	97.4 ± 2.0	14.3 ± 8.7
Ca		87.8 ± 7.3	87.6 ± 7.3[a]	85.3 ±4.6	83.8 ±5.0[ab]	82.0 ± 6.5	80.9 ± 6.2[b]
P		95.5 ± 3.2	73.5 ± 6.4	92.6 ±1.2	67.7 ±4.9	94.9 ± 3.1	70.9 ± 5.3
Mg		36.2 ±10.2	24.9 ± 5.3	41.2 ±6.9	30.4 ±5.3	41.0 ±10.9	24.4 ± 7.5

[1] Treatment C lasted 3 days instead of the 5 days intended.
[2] Mean ± sd; means not sharing a common letter differ significantly between treatments (F-test).
[3] Number of samples; *N* is *Normal*, *L* is *Loose* and *D* is *Diarrhoea*.
[4] Apparent faecal absorption coefficient (in % of oral intake).
[5] Retention coefficient (in % of oral intake).

though they proved to be significant. These regressions are therefore only used to give a general impression of the effect of dietary lactose on the average changes in the apparent faecal absorption and the retention coefficients of Na and K in this experiment. An increase of dietary lactose with 1 g Hex. Eq. per kg BW per day reduced the average absorption coefficient in the small intestine by 4.6 and 1.0 units for Na and K, respectively. The average faecal absorption coefficient declined by 2.2 and 1.4 units and the retention coefficient by 4.1 and 1.7 units, respectively.

The changes in electrolyte absorption, measured in our experiment, were less serious than those cited in the literature as occurring in calves suffering pathogenically induced scours. E.g. FISHER et al. (1972) measured in calves suffering severe scours additional losses of 12 and 9 mg per kg BW, respectively. These losses increased to 70 and 35 mg per day and per kg BW in calves dying of scouring. In our experiment treatment C increased the daily faecal excretion of Na and K by approximately 8 and 16 mg per kg BW, respectively, compared with treatment A. Obviously, lactose diarrhoea lasting for 3 to 5 days is less serious in this respect. Nevertheless the effect of the dietary treatments cannot be neglected. In particular, one has to be aware of the large differences in individual responses. In this experiment one calf lost 14% more Na than ingested.

In summary it is clear that high levels of dietary lactose do not result in a significantly higher total water excretion in faeces plus urine. The extra faecal water excretion is compensated for by a reduced urine excretion. With high lactose intakes carbohydrate excretion in the urine is significantly higher than in faeces.

The absorption and retention of Na and K are decreased by the high lactose treatments and those of Ca and Cl may be affected as well. Most animals did not suffer net electrolyte losses in treatments B and C, but it was observed for Na in some individual calves.

5.7. GENERAL DISCUSSION AND CONCLUSIONS

The results in Exp. 6–15 provide convincing evidence that at high intakes of dietary lactose by milk-fed calves the changes in intestinal digesta and faecal consistency are basically identical with the disturbances observed in man suffering hypolactasia. Both disturbances are caused by an increased amount of non-digested, and/or non-absorbed carbohydrates in the intestinal lumen and, consequently, a reduced water absorption. These symptoms are usually aggravated by enhanced microbial fermentation of the carbohydrates in the lower intestine, which results in more osmotically active substances in the lumen and a further reduction of the water absorption. However, in calves the enzymatic activity of the lactase is not primarily responsible for putting a limit on the lactose intake.

Duodenal flow rates of wet digesta are not affected by the lactose treatment, nor those of other milk components, e.g. nitrogen and NFE (Exp. 9). Abomasal

transit time obviously is not a factor in reducing the apparent ileal and faecal digestibility of the lactose fraction.

The amount of lactose apparently digested and absorbed in the small intestine increased substantially with lactose intake (Exp. 9, 12, 13). It confirms the general view that hexose transport across the duodenal mucosal wall increases with its concentration in the lumen. The increase in sugar absorption does not, however, prevent greater amounts of carbohydrates flowing into the hind gut. The results in Exp. 13 and 14 support the opinion that the absorption of galactose is inhibited by glucose in the small intestine of calves (COOMBE, 1973; GROPP, 1973). The competition between both sugars in the transport mechanism becomes more apparent when lactose intake increases. In those conditions the apparent glucose digestibility in the small intestine seems not to be greatly affected. The apparent digestibility of galactose at that site is, however, substantially reduced by the higher level of dietary lactose.

In the literature the lactase activity in the brush border was frequently stated to impose restrictions upon the level of dietary lactose tolerated by milk-fed calves. That conclusion may in some occasions have been based upon an inaccurate lactose analysis, as is shown in Exp. 13 and 14. The analysis, based upon enzymatic carbohydrate digestion and assuming lactose to be the only galactose containing sugar in that material, overestimates the lactose content in ileal digesta and faeces. The conversion of lactose into other di- and trisaccharides by transgalactosylation in the brush border was apparently not foreseen by the authors as occurring in milk-fed calves.

The role of the oligosaccharides in the digestive and absorptive process of lactose in these animals is not yet clear. In-vitro experiments showed that these components are less easily digested by β-galactosidase than lactose. An aqueous solution of lactose was completely hydrolysed within 20 minutes; in that time the galactose, derived from hydrolysis in samples of ileal digesta, averaged ca. 78 % of that measured after an overnight incubation. Some oligosaccharides seem to be rather resistant to β-galactosidase digestion. However, their higher resistance to lactase digestion does not reduce the efficiency of glucose and especially galactose absorption in the small intestine. The apparent ileal digestibility of galactose proved to be higher in treatment C, when the dietary lactose was partly converted into oligosaccharides, than in treatment C′, offering almost exclusively glucose and galactose and inducing hardly any conversion in the small intestine (Exp. 14). Neither did these oligosaccharides seem to be responsible for the preferential absorption of glucose compared to that of galactose. The difference between the quantities of glucose and galactose in ileal digesta was greater when the free hexose diet C′ was fed. These results support the opinion that the hexoses resulting from hydrolysis in the brush border are more rapidly transported across the mucosal wall than free hexoses in the intestinal lumen (CZÁKY, 1975).

The shorter transit time in the abomasum plus small intestine, observed in treatment C′, may also have influenced the hexose absorption in that treatment (figure 16). One might speculate that in treatment C (lactose) initially the milk sugar and, in the distal part of the small intestine, the oligosaccharides have

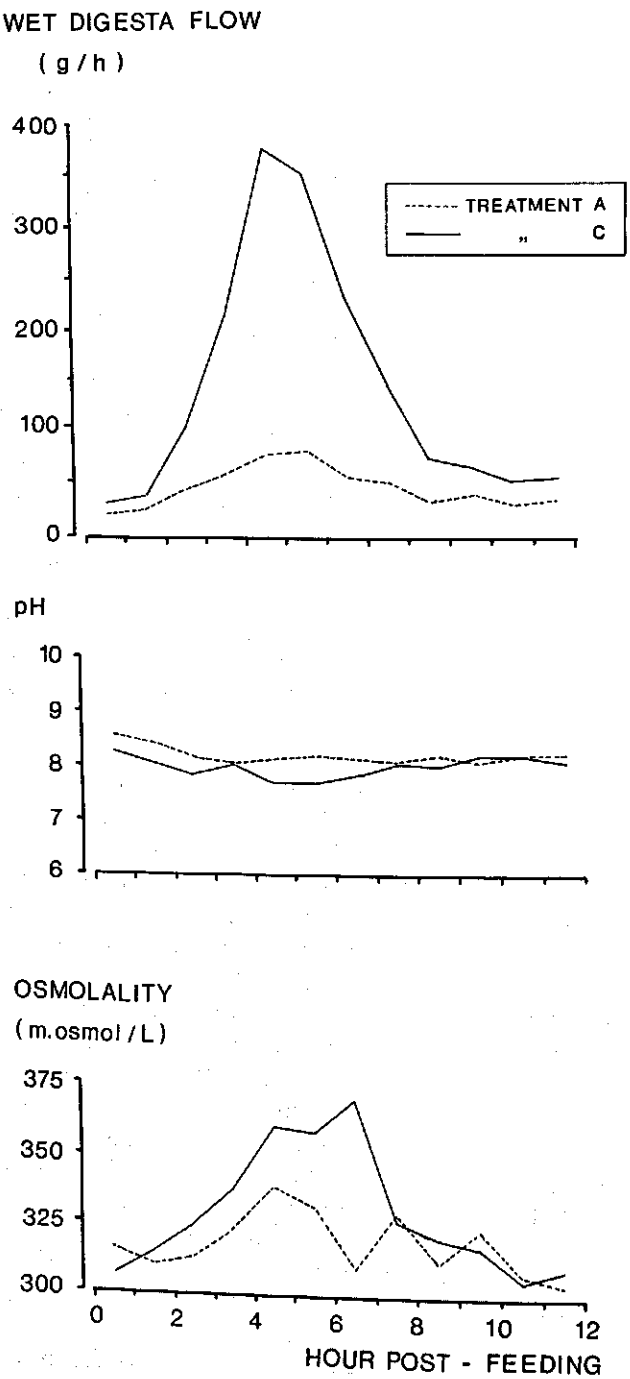

FIG. 20. The effect of dietary lactose on ileal flow of wet digesta and digesta pH and osmolality (Exp. 11).

permitted greater absorption of water from the lumen, because of their lower osmotic effects compared with those of the free hexoses in treatment C'. The average recovery of wet digesta was, in fact, approximately 12 units lower in treatment C, although that difference was not significant (table 28, P_2/P_4). According to the literature such a difference may also have been the cause of the longer transit time of the lactose diet and hence beneficial too in the absorption of hexoses in that treatment.

When diarrhoea is defined as a disturbed water absorption, it occurred in our study already in the small intestine of the calves, receiving high levels of dietary lactose. In these circumstances, the digesta flowing into the lower intestine was characterized by higher amounts of water, sugars and minerals, particularly Na and K. Digesta osmolality at the distal ileal site, systematically investigated in 34 calves used in the separate experiments, was not affected by the lactose treatment. It averaged 312 $\pm$ 12 m osmol per L, irrespective of lactose intake. Occasionally the data seemed to indicate slightly higher values in treatment C when ileal flow rate was high (figure 20). These differences, however, were not consistent in the experiments and seemed to be of minor importance.

The results in Exp. 3, 5 and 15 show that the macro elements in ileal digesta are primarily responsible for digesta osmolality, in particular Na and K. In Exp. 15 these two cations, together with their respective anions, were responsible for about 46 % of the total osmolality in ileal digesta (312 m osmol/L). In treatment C their relative importance in total osmolality decreased to approximately 38 %. Based on the free glucose and galactose content in ileal digesta, measured in Exp. 13 and 14, the relative osmotic activity of the free hexoses increased from about 2.4 to 27 % in treatments A and C, respectively. The average increase in cumulative wet digesta flow was about 1.5 L in the latter treatment, while osmolality was not different in either treatment. These results indicated that approximately 60 % of the increase in digesta volume was attributable to the osmotically active free galactose. Na and K, with their associated anions, accounted to the extent of approximately 28 % for the greater volume of digesta flowing into the hind gut. The absorption of Ca in the small intestine was affected to some extent by the high lactose diet, but this would have had little osmotic effect there (SMITH, 1966; VAN 'T KLOOSTER, 1967). The higher amounts of carbohydrate were therefore mainly responsible for the additional osmotic activity and hence for the increased ileal flow rate of wet digesta.

The changes in ileal digesta are responsible for the faecal disturbances (Exp. 8, 11). Although their effect upon the chemical and microbial processes in the hind gut was not investigated, the results may indicate that osmotic effects, similar to those observed in the small intestine, occur also in the colon. The alkali metals Na and K are rather efficiently absorbed in the colon (Exp. 15). The relative importance of these minerals in colonic osmolality will therefore steadily decrease. The increase in easily fermentable carbohydrates flowing into the hind gut stimulates microbial fermentation. In particular, considerable amounts of lactic acid are produced in colonic digesta (CLARKE et al., 1977; VAN WEERDEN, pers. comm.). Although FFA absorption occurs (XENOULIS, 1967), its extent was

not sufficient in our experiments to compensate for the increased amount of osmotically active substances and allow normal water absorption, thus resulting in a higher water excretion and a reduced faecal pH. Whether or not lactic acid decreases digesta transit time in the hind gut was not investigated in our experiments.

The transgalactosylation of lactose may also have some benefits in reducing scouring, as is shown by the carbohydrate composition in ileal digesta and faeces in treatments C and C′. In the former treatment only 18% of the total hexose content in ileal digesta consisted of the free hexoses, glucose and galactose; 82% was bound in oligosaccharides. These proportions were 88 and 12%, respectively, in the latter treatment. The osmotic processes in the colonic lumen will have been significantly affected by these differences, not only because of the higher quantity of free hexoses, but also by their greater osmotic activity compared with the oligosaccharides. Moreover, the resistance of the oligosaccharides to β-galactosidase digestion, as was observed in the in-vitro experiments, may also have influenced the microbial fermentation. The slightly lower apparent faecal digestibility of the carbohydrates in diet C may give some support to that supposition.

Not only were there large differences in response by individual calves, but there were also seasonal differences. This made our results scarcely sufficient to estimate accurately the acceptable limit of lactose tolerance of the young calf. The individual differences were considerable and seemed to be reflected in each

TABLE 36. The Na absorption and retention in some individual calves on treatments A and C (Exp. 15).

	Calf 12[1]		Calf 15[2]	
	Treatment A	Treatment C	Treatment A	Treatment C
Faecal characteristics:				
Visual score[3] *N*	9	1	7	–
L	–	11	–	4
D	–	4	–	31
pH	6.8 ±0.3[4]	4.6 ±0.8	7.3 ±0.4	4.1 ±1.0
Faecal excretion (g/day)	297.8	1803.0	309.2	6942.0
Apparent faecal Na absorption coefficient (% of oral intake)	98.7	93.5	99.5	68.4
Na retention coefficient (% of oral intake)	36.0	30.2	34.2	–14.3

[1] The oral intake during faecal collection was 10.3 ±0.2 and 17.0 ±0.5 g Hex.Eq. per kg BW per day in treatments A and C, respectively.
[2] The oral intake during faecal collection was 10.3 ±0.2 and 17.1 ±0.1 g Hex. Eq. per kg BW per day in treatments A and C, respectively.
[3] Number of samples scored *Normal (N)*, *Loose (L)* or *Diarrhoea (D)*.
[4] Mean ± sd.

parameter measured in our study, as is illustrated in table 36 by the difference in Na absorption and retention (Exp. 15). Calves, that respond quickly to the dietary treatments, have to be considered as standard for maximal lactose tolerance, because nutritional diarrhoea is not allowed to occur. That minority of animals suggests that it would be inadvisable to exceed the lactose intake used in control diet A, i.e. 10 g Hex. Eq. per kg BW per day in calves older than four weeks.

Our work clearly indicates that milk sugar is the only carbohydrate efficiently digested and absorbed in milk-fed calves. It is also the only one that is tolerated in considerable amounts. Neither the addition of other carbohydrates e.g. starch, oligosaccharides or hexoses to that maximal amount, nor pre-hydrolysis or inclusion of carbohydrate splitting enzymes will increase that acceptable limit of carbohydrates that these animals can tolerate.

SUMMARY

Diarrhoea is a serious disturbance of normal gut function, characterized by an excessive water excretion in faeces. The derangement is in particular observed in young, milk-fed calves. In the literature a distiction is usually made between scouring from nutritional factors and that caused by pathogenic infections. The former derangement in calves is investigated in our work.

In practice it is well known that the new-born calf is highly susceptible to nutritional diarrhoea. Its prevention requires a number of precautions in calf nutrition and management. Nowadays these requirements also impose severe restrictions upon the type and amount of feed components suitable for use in milk replacers for these animals. Nevertheless their importance in calves, the role of diet composition and intake in this disturbance has not been systematically investigated. The lack of information prompted an investigation of the role of diet composition and intake in scouring disturbances in milk-fed calves. The work included experiments, where the main objective was to classify common diet components according to their diarrhoeic properties. The changes in the digestive processes were also measured. Associated changes in the microbial population in the lower intestine, and in electrolyte and water metabolism in body tissues were not investigated.

The literature on the physiological changes responsible for nutritional diarrhoea in calves, reviewed in chapter 2, is rather scarce and generally refers to research in infants and man. According to these investigations proteins and carbohydrates are considered to have the greatest potential to induce scouring. Exceeding the digestive capacity of these components in the small intestine may result in either proteo-saccharolytic or saccharolytic fermentation in the hind gut. Both conditions inhibit water absorption in the colon, either because of an increased amount of components with osmotic activity in the digesta, or because of toxic properties in the end products of fermentation. Certain products of proteo-saccharolytic fermentation in particular are considered to be toxic. The derangements may lead to either putrefactive diarrhoea when dietary protein is involved, or saccharolytic diarrhoea in case of excessive carbohydrate intake. These two types of scouring are among others characterized by changes in faecal pH, being higher or lower, respectively, than in normal faeces. The literature on these effects in milk-fed calves is less clear. It provided no insight into the different diarrhoeic properties of the individual organic components, protein, fat or carbohydrates, in the diet. Another question arising was the role of abomasal clotting of the diet in scouring. This was by several authors considered to be important in putrefactive diarrhoea.

Five experiments were carried out to investigate these aspects in young, milk-fed calves. In the first experiment (Exp. 1, section 3.3) the effect of milk composition and daily allowance on faecal characteristics (visual score, pH and DM content) was investigated in twelve subsequent periods, each lasting three

days. A commercial milk replacer served as control diet in six calves, providing approximately 4.5 g protein, 3.5 g crude fat and 8 g Hex. Eq.*) lactose per kg BW per day. In the separate experimental periods either lactose, sucrose, gelatinized or raw starch, casein or fat was added in addition to that diet. The extra lactose addition to the control diet varied up to 125 % of control Hex. Eq. intake, sucrose and starch to 75 %, casein to 140 % of control crude protein intake and fat to 60 % of control crude fat intake. In one period the daily offer of the control diet was increased to 175 % of the normal intake.

The carbohydrate additions resulted in a rapid fall of faecal consistency and pH (table 2). Sucrose and the starches proved to be more critical in this respect than lactose, although the response to starch was slightly delayed. Dietary lactose induced quickly fermentative scouring when fed in excess of 10 g Hex. Eq. per kg BW per day. Calf's response to lactose was not affected by animal's age in this experiment, contrary to that reported by several authors. High concentrations of well homogenized fats of high nutritional quality seemed to become critical too, although the effect was less pronounced than with similar increases of carbohydrates (table 3). Scouring was also observed on high milk intakes (table 4). The response of faecal pH and DM content in this period suggested an interaction in scouring effects between the dietary components. The experiment did not allow any conclusion to be drawn as to whether or not the interaction interfered with the digestion or the microbial fermentation of the undigested dietary residues in the lower intestine. Casein did not affect faecal characteristics, irrespective of the level of intake.

Although faecal responses to the individual treatments were evident, they were less informative about the quantitative differences in diarrhoeic properties of the individual components tested. Faecal pH was usually closely related to visual score when increasing carbohydrate intake. This parameter, however, gave less information on the effect of high fat or protein intake. A diagnosis based on DM content alone might lead to misinterpretation of the severity of scours, as was demonstrated in the starch treatments. This criterion does not always reflect the increased water excretion in diarrhoeic faeces. It may be affected simultaneously by a higher excretion of components exerting no osmotic effects, e.g. mucous substances.

The absence of scouring in the high casein treatments seemed to conflict with the results cited in the literature. These postulate the risk of putrefactive scouring induced by dietary protein, in particular, when milk clotting in the abomasum is insufficient. The role of abomasal clotting in diarrhoea was therefore investigated in Exp. 2 (section 3.4). For that purpose a liquid commercial milk replacer was directly infused into the proximal duodenum, replacing quantitatively the digesta collected at that site. The effect of this treatment on faecal characteristics, visual score and pH, was measured in two experimental periods lasting 4 × 12 h and 4 × 24 h, respectively.

The milk infusate increased substantially the firmness of the faeces, especially

*) See page 16.

in the latter period (table 5). Faeces became so firm that the animals had obviously problems in defaecating. This unexpected, and yet still unexplainable, result clearly shows that no negative relation exists between abomasal clotting of milk protein and scouring.

The importance of undigested dietary components in the large intestine digesta in the scouring derangement was more closely investigated in Exp. 3, 4 and 5 with fifteen ileal fistulated calves (section 3.5). The higher inflow of dietary components into the colon was simulated by an infusion of graded levels casein, fat or lactose into the distal end of the ileum. Casein was infused at 5 and 10 % of oral crude protein intake. The fat infusion was 5 % of crude fat intake and those of lactose 5, 10 and 20 % of oral NFE intake in the control milk replacer. Faecal response (visual score, pH and DM content) was measured, as well as the ileal and faecal apparent digestibility.

The effect of the infusates on faecal characteristics (table 6) merely confirmed those observed in Exp. 1, although faecal responses were rather mild. The casein and fat treatments did not change faecal characteristics. Only the highest level of lactose infusion, 20 % of oral NFE intake, affected all faecal parameters. The control diet was highly digestible in these experiments (table 7). The digestion and absorption of most dietary components was almost completed at the distal end of the small intestine. Only a minor part of N and NFE disappeared in the lower intestine together with a substantial amount of Na. Faecal digestibility was hardly affected by the casein and fat infusates (table 8). N and NFE excretion in the faeces increased, however, when the lactose infusion exceeded 5 % of oral NFE intake. Faecal N excretion increased by 15–16 mg per g lactose infused in addition to the 5 % treatment.

The results of Exp. 1–5 indicate that high intakes of milk protein of standard quality, i.e. casein, are not detrimental to young calves. High intake of fats or carbohydrates may result in nutritional scouring. The fat concentration of the diet is, however, presumably more limited by technological capabilities than by the level tolerated by these animals. It was therefore decided to undertake further work on the effect of carbohydrates. As lactose is the most important carbohydrate in milk replacers, this sugar received our main attention.

The literature on the digestion and absorption of carbohydrates (chapter 4) indicates that milk-fed calves have an impressive ability to digest lactose and to absorb glucose and galactose. Consequently their levels of intake permitted in these animals are rather high. The enzymatic activity for digestion of other carbohydrates is much less well developed or even absent. Their intake is therefore strictly limited or even not tolerated at all. The literature is less informative about the digestive changes in young calves responsible for the nutritionally induced scouring. Neither have the consequences of the derangement for animals' health been investigated systematically. Four important questions in this respect needed further clarification. They concerned: (1) the maximal limit of lactose permitted in milk replacers for young calves, (2) the effect of excessive lactose intake on the digestion and absorption in the small intestine, (3) the significance of the quantitative and qualitative characteristics of ileal digesta in

scouring and (4) the effect of fermentative diarrhoea on the water and mineral excretion. These aspects were investigated in ten experiments (Exp. 6–15, Chpt. 5).

Exp. 6 and 7 were designed to investigate the relationship between lactose intake, carbohydrate digestion and absorption, using jugular vein blood sugar as a parameter, and scouring in young calves. In the former experiment graded levels of dietary lactose were fed to thirty calves, allotted to five groups of six animals. The individual faecal responses were measured and related to the individual blood sugar responses. Lactose was offered in three treatments, A, B and C, supplying 10, 13.5 and 17 g Hex. Eq. per kg BW per day, respectively. All treatments were fed for three successive days in each period. In Exp. 7 treatment A and C were offered to nine calves. The experimental periods were prolonged in this trial from three to seven days in order to test the possible effect of adaptation to high lactose intakes.

The results in both experiments confirm that fermentative scouring quickly occurs, when the daily lactose intake exceeds 10 g Hex. Eq. per kg BW in calves aged at least four weeks. The response in blood sugar level provided evidence that the digestion and absorption of lactose increase with the daily intake. Treatment C resulted in significantly higher blood sugar levels than treatment B; those were in both treatments significantly higher than in control treatment A (table 14, 16). The blood sugar responses seemed also to indicate that calves, responding in a less pronounced way regarding the blood sugar curve in treatments B and C, suffer scouring more seriously. No adaptive response in the blood sugar curve occurred when the high lactose treatment was prolonged to seven days. However, calves adapt quickly to high lactose intake in their first four weeks of life. No effect of age was demonstrated from 4–12 weeks of age on the limit of lactose tolerance. The slope of the blood sugar curve, however, becomes steeper as animals grow older.

The effect of dietary lactose on the digestive processes in the small intestine was further investigated in six experiments. Digesta flow rate*), transit time*) and digesta osmolality were the main parameters investigated in Exp. 9, 10 and 11 (section 5.4.1). In Exp. 9 sixteen calves were used for this purpose, either fitted with re-entrant duodenal or ileal cannulae, or not fistulated. The effect of dietary lactose was tested in treatments A and C, offering 10 and 17 g Hex. Eq. lactose per kg BW per day, respectively. The duodenal or ileal flow rates of wet digesta, N and reducing substances were measured in the fistulated calves. The non-fistulated animals served as a 'control group' to test the scouring effect of the dietary treatments in this experiment. The flow rates of ileal wet digesta, measured in Exp. 10, 11 and 13, closely agreed with those observed in Exp. 9. In these trials treatment A and C were tested and also treatment D, offering 8 g Hex. Eq. lactose and 3 g Hex. Eq. sucrose per kg BW per day.

The scour-inducing level of lactose (treatment C) neither affects abomasal transit time or duodenal flow rates of wet digesta, nor that of its components, N

*) See page 51.

and reducing substances (table 19). Neither the digesta transit time, nor N flow rates are different at the end of the ileum for either treatment (table 29). The flow rates of wet digesta and reducing substances are, however, substantially higher when daily lactose intake increases from ca. 9–10 to ca. 16–17 g Hex. Eq. per kg BW. Treatment D, supplying sucrose, basically acts in scouring in a similar way as lactose, although the calves respond more seriously to this treatment (table 21). Compared with the lactose diets, wet digesta flow rates into the lower intestine are significantly enhanced and digesta transit time in the abomasum plus small intestine is reduced in the sucrose treatment.

The effect of high lactose intake on the apparent digestibility of dietary components in the small intestine was measured in Exp. 12 (section 5.4.2) On account of the interaction observed in Exp. 1 the experiment was designed to investigate also the influence of diet composition on the digestion. Fifteen calves, fitted with re-entrant ileal cannulae, were allotted to three groups, receiving either diet A, B or C in five experimental periods of five days each. In P_1 and P_5 the apparent digestibility of the diets, offered in amounts equal to those usually given in treatments A, B and C, was investigated. In the other periods the animals received equal amounts of lactose with the three diets; 15.3, 12.2 and 9 g Hex. Eq. per kg BW per day in P_2, P_3 and P_4, respectively.

The feed intake and digestive ability of the calves declined when the experiment lasted longer. This prevented an accurate determination of the apparent digestibility. Covariance analyses provided much evidence that the apparent digestibility coefficients of the main dietary components in the small intestine are hardly affected by lactose intake. Only the carbohydrate fraction is relatively less efficiently digested when lactose intake increases (table 22). It also seems to reduce the apparent digestibility of crude protein. It confirmed our view that, although the lactose digestion and absorption improves in the high lactose treatments, the improvement is insufficient to prevent greater amounts of carbohydrates flowing into the hind gut. The apparent digestibility in the small intestine is not substantially affected by diet composition. The differences in Exp. 12 were too small to be responsible for the large effect of diet composition on faecal characteristics on high milk intake in Exp. 1. These latter differences were obviously caused by differences in microbial fermentation in the lower intestine. The level of lactose that can be tolerated is obviously an absolute amount depending upon body weight, not on the percentage composition of the diet.

In two subsequent trials, Exp. 13 and 14, the significance of lactase activity in the maximal limit of lactose intake was investigated (section 5.4.3). The previous experiments give little information on that aspect, especially because of a discrepancy between the results obtained in analysis of reducing substances and of the individual sugars, glucose, galactose and lactose, according to a standard enzymatic method. Exp. 13 was designed to get more information on that point and to determine more accurately the individual sugar content in ileal digesta. For that purpose diets A and C were fed to five calves, fitted with re-entrant ileal cannulae. Ileal digesta and faeces were quantitatively collected in separate experimental periods.

The results (table 24, 25 and 26) suggest that glucose is efficiently absorbed, mainly in the small intestine. In agreement with the literature, absorption of galactose is reduced in that region, in particular when lactose intake increases. Lactose recovery in ileal digesta and faeces is low compared with galactose recovery. The analyses on carbohydrate composition strongly suggested that the major part of galactose in ileal digesta was a component of other oligosaccharides, containing approximately 4.5 to 4.6 times as much galactose as glucose molecules. These compounds would explain the observed discrepancies between reducing substances and the individual sugars analysed, and also the lower efficiency of β-galactosidase in the 'lactose' digestion in ileal and faecal samples. The fact that these compounds were not found in the milk diet or duodenal digesta strongly suggested that they were synthesized in the small intestine during lactose digestion.

Whether or not lactose is converted by lactase into oligosaccharides in calves, was furher investigated in Exp. 14. In this trial the high lactose diet C and a high hexose diet C′ were fed. Both diets offered daily equal amounts of Hex. Eq.; 16 to 17 g per kg BW. The diets were fed to ten calves, fitted with re-entrant ileal cannulae and to eight non-fistulated calves. Ileal digesta and faeces of the fistulated animals were quantitatively collected and analysed by gas chromatography for sugar content. The non-fistulated calves served as a 'control group' to measure the scouring response of the dietary treatments more accurately.

Both diets induced scours in the calves (table 27). Treatment C′ seemed to be slightly more conducive to diarrhoea and resulted in a weak condition of the calves, probably resulting from lesions in the intestinal mucosa. These adverse effects still carried over when this treatment had been stopped for three weeks and replaced by control diet A. The difference between both treatments was also reflected in the flow rates of ileal wet digesta, being higher in treatment C′ than in the lactose treatment C (table 28).

The sugar analyses in ileal digesta, collected in treatment C, proved that substantial amounts of hexoses were bound as oligosaccharides, i.e. lactose, maltose and other di- and trisaccharides, mainly consisting of galactose molecules. The samples collected in treatment C′ contained more free and total glucose and galactose, but hardly any oligosaccharides. The recovery of total glucose and galactose in both treatments confirms the preferential absorption of hydrolysed hexoses over free hexoses in the intestinal lumen.

Free glucose and galactose were the main sugars excreted in the faeces (table 29). Traces of lactose and other disaccharides were only found in faeces collected in treatment C, but not in treatment C′. Trisaccharides were not excreted in the faeces. The recovery of total glucose and galactose in the faeces, although almost negligible in both treatments, was slightly higher when lactose was fed.

The results in Exp. 13 and 14 confirm the conversion of lactose into other oligosaccharides by lactase in the small intestine of calves. These compounds are not responsible for the lower galactose absorption in relation to glucose absorption. The apparent digestibility of galactose in the small intestine is slightly higher when lactose is fed instead of the individual hexoses, glucose and galac-

tose. They also confirm that the limits put on galactose absorption are mainly responsible for maximal lactose digestion and absorption. Although some lactose is recovered at the end of the small intestine, this quantity is much lower than the total galactose recovery. Moreover, the lactose in ileal digesta may to some extent originate from transgalactosylation. The total galactose content is the main factor responsible for the changes in ileal digesta, when diets high in lactose are given to young calves.

The effect of the quantitative and qualitative characteristics of ileal digesta in scouring was investigated in two trials (Exp. 8 and 11). In the latter experiment ileal digesta were quantitatively collected in calves, receiving the sucrose treatment D, and infused into the distal ileal cannula of calves, receiving control diet A, and vice versa. The three calves showed severe scouring when infused with ileal digesta from diet D (table 30). The other three calves, receiving the diet A infusate, responded with normal faecal characteristics despite the sucrose intake. The results confirmed that the changes in ileal digesta, when feeding an excess of carbohydrates, are responsible for the scouring phenomenon.

The carbohydrate content in ileal digesta is generally presumed to be the main factor in scouring. However, the response of calves to carbohydrate infusions at that site were rather mild in Exp. 5 in relation to those usually observed in treatment C. It prompted us to repeat that trial, infusing lactose and galactose in amounts equal to 40 % of oral Hex. Eq. intake (Exp. 8). All sugars were infused as an aqueous solution, isotonic with ileal digesta (312 m osmol per L). An isotonic NaCl solution served as a control treatment in this experiment.

The introduction of carbohydrates into the colonic lumen quickly reduced faecal consistency, pH and DM content (table 31). The responses were almost similar to those in calves severely suffering scours on high dietary lactose intakes and indicate that the carbohydrates in ileal digesta are those primarily responsible for the scouring.

The effect of lactose induced scouring on the water excretion of the calves was investigated in Exp. 9, 14 and 15, measuring quantitatively the urine and the water excretion in faeces in treatments A, B and C (section 5.6). The results suggest that lactose induced scouring in milk-fed calves does not necessarily change the total water excretion (table 32). The extra faecal water loss is compensated for by a lower urine excretion. The carbohydrate excretion in the urine is, however, significantly increased in high dietary lactose intake (table 33).

The effect of dietary milk sugar in diets A, B and C on the absorption and excretion of macro minerals in milk-fed calves was investigated in Exp. 15. These diets were fed in three successive periods of five days each to twenty calves, ten of them fitted with re-entrant ileal cannulae. The absorption of Na, K, Cl, Ca, P and Mg in the small intestine was investigated in the fistulated calves. The non-fistulated animals served to measure the faecal apparent absorption and the retention of these minerals.

The apparent absorption of the minerals in the small intestine in treatment A was rather high (table 34). The results in that treatment agree with those cited in the literature. The absorption of most elements is almost completed in the small

intestine, except of Na, which is absorbed in considerable amounts in the large intestine. The absorption in the small intestine of K, Cl, Ca and in particular of Na decreases when dietary lactose intake increases. This effect is also reflected in the apparent faecal absorption and in the retention of these minerals, but only the changes in the absorption and retention of Na and K were significant in this experiment (table 35). Most animals did not suffer net electrolyte losses in treatment B or C. However, that did occur with Na in some individual calves, that responded severely to the lactose treatments (table 36).

The results obtained in these fifteen experiments prove that lactose is the only carbohydrate tolerated in considerable amounts in milk replacer diets by young calves. The maximal limit of lactose, ca. 10 g Hex. Eq. per kg BW per day, seems to be slightly higher than that of glucose and galactose. It is not yet clear, whether or not the oligosaccharides in the ileal digesta, originating from the conversion during lactose digestion, may have some benefits in this respect. When, however, the lactose intake exceeds the limit of hexose, and in particular that of galactose absorption, scouring occurs. Related changes in intestinal digesta and faecal characteristics are basically similar as those observed in infants and man, suffering hypolactasia.

SAMENVATTING

Diarree is een ernstige verstoring van de normale darmfunctie, gekarakteriseerd door een abnormaal hoge wateruitscheiding met de mest. Vooral jonge kalveren, die (bijna) uitsluitend melk krijgen, lijden dikwijls aan deze stoornis. De literatuur maakt meestal een onderscheid tussen twee belangrijke oorzaken voor diarree: de voeding en pathogene infecties. Ons onderzoek richtte zich uitsluitend op het verband tussen de voeding en het optreden van diarree bij kalveren.

Van oudsher is bekend dat het jonge kalf zeer gevoelig is voor voedingsstoornissen, resulterend in diarree. De preventie vereist een groot aantal voorzorgen in de dagelijkse voeding en verzorging van deze dieren. Tegenwoordig stellen deze eisen tevens kwalitatieve en kwantitieve grenzen aan de voedermiddelen die in kunstmelk voor kalveren kunnen worden gebruikt. Ondanks het feit dat voedingsdiarree een belangrijke factor is in de opfok en het mesten van kalveren, is weinig systematisch onderzoek verricht naar de rol die de voersamenstelling en -opname hierbij spelen. Het gemis aan informatie vormde de aanleiding tot dit onderzoek. Het beschreven werk betreft een aantal proeven, waarin werd getracht de gebruikelijke voedermiddelen in kunstmelken te classificeren op basis van hun diarree-verwekkende eigenschappen. Tevens werden de veranderingen in het verteringsproces die bij een extreem hoge opname van deze componenten optreden nader onderzocht. De daarmee gepaard gaande wijzigingen in de microbiële populatie in de blinde- en dikke darm, of die in de electrolyten en waterhuishouding in lichaamsweefsels werden niet rechtstreeks onderzocht.

De literatuur over de fysiologische oorzaak van voedingsdiarree bij kalveren (hoofdstuk 2) is beperkt en verwijst meestal naar de inzichten gebaseerd op humaan-medisch onderzoek. Volgens die literatuur vragen vooral de eiwitten en koolhydraten bijzondere aandacht in verband met hun laxerende eigenschappen. Indien de opname van deze bestanddelen de maximale resorptie in de dunne darm overschrijdt, zal in het achterste deel van het darmkanaal een proteo-sacharolytische en/of een sacharolytische fermentatie worden gestimuleerd. Beide vormen van microbiële fermentatie remmen bij de mens de waterresorptie; enerzijds als gevolg van een toename van osmostisch actieve bestanddelen in de chymus en anderzijds door toxische eigenschappen van eindprodukten van de fermentatie. De nadelige invloed van de eindprodukten van de eiwitfermentatie op de darmmucosa worden in dit verband benadrukt. Bovengenoemde verstoringen leiden tot „rottingsdiarree" indien te veel eiwitten, of tot „fermentatieve" diarree ingeval te veel koolhydraten worden verstrekt. Beide vormen van diarree onderscheiden zich onder meer door de pH in de faeces, die in rottingsdiarree hoger en in fermentatieve diarree lager is dan normaal.

Of, en in hoeverre deze verbanden ook gelden in voedingsdiarree bij kalveren was minder duidelijk. De literatuur gaf weinig inzicht in verschillen tussen de organische bestanddelen, eiwitten, vetten en koolhydraten met betrekking tot

hun maximaal toelaatbare grenzen met het oog op de voedingsdiarree. Een tweede vraag in dit verband betrof de functie van de stremming van melkeiwit in de lebmaag. Verschillende auteurs vermeldden een nauw verband tussen de strembaarheid van het voeder en het optreden van rottingsdiarree.

In een vijftal proeven werden deze aspecten nader onderzocht. In de eerste proef (Exp. 1, paragraaf 3.3) werd het effect gemeten van de samenstelling en opname van het voer op de eigenschappen van de mest, gekarakteriseerd door een visuele beoordeling van de consistentie, door de pH en het ds-gehalte. Hiertoe werd een handelsvoeder verstrekt aan zes kalveren in twaalf opeenvolgende proefperioden van drie dagen. De dagelijkse dosering van dit controlevoer bedroeg 4,5 g re, 3,5 g rvet en 8 g Hex. Eq.*) lactose per kg LG (lichaamsgewicht). Dit rantsoen werd in de verschillende proefperioden aangevuld met koolhydraten (lactose, sacharose en wél of niet verstijfseld zetmeel), caseïne of vetten. De extra lactose toevoeging bedroeg maximaal 125 % van de hoeveelheid Hex. Eq. verstrekt met het controlevoer, die van de andere koolhydraten maximaal 75 %, die van caseïne maximaal 140 % van de controle re-dosering en die van het vet 60 % van de normale rvet-dosering. In één proefperiode werd de dagelijkse verstrekking van het controlevoer verhoogd met maximaal 75 % van de normale dosering.

De hogere opname van koolhydraten resulteerde snel in een daling van de mestconsistentie en pH (tabel 2). Sacharose en de zetmelen bleken in dit verband riskanter te zijn dan lactose, al trad de reactie op zetmeel iets later op dan die op de disachariden. Lactose veroorzaakte diarree indien de dagelijkse opname hoger was dan 10 g Hex. Eq. per kg LG. De laxerende werking van melksuiker bleek niet afhankelijk te zijn van de leeftijd van de kalveren in deze proef. De verhoging van het aanbod van goed gehomogeniseerde vetten van goede kwaliteit leek eveneens tot een verminderde mestconsistentie te leiden. De daarmee samenhangende veranderingen in de mest waren echter minder duidelijk dan die bij vergelijkbare verhogingen van de koolhydraten (tabel 3). Indien de melkopname werd verhoogd werden eveneens stoornissen opgemerkt, resulterend in een verminderde mestconsistentie (tabel 4). De reactie van de pH en het ds-gehalte in de mest leek bij deze proefbehandeling te wijzen op een interactie tussen de individuele melkbestanddelen in relatie tot hun diarree-verwekkende eigenschappen. De proef gaf geen inzicht of deze interactie optrad tijdens de vertering in de dunne darm, of bij de fermentatie in de dikke darm. De toevoeging van caseïne had in dit onderzoek geen invloed op de eigenschappen van de mest.

Hoewel de meeste behandelingen in deze proef de mestconsistentie duidelijk beïnvloedden, gaven de waargenomen veranderingen weinig inzicht in de ernst van de diarree. De maatstaven die werden gebruikt om deze verschillen vast te stellen waren slechts gedeeltelijk informatief. De pH van de mest lijkt bruikbaar te zijn wanneer fermentatieve diarree optreedt bij hoge koolhydraten verstrekking. Een classificatie, uitsluitend gebaseerd op het ds-gehalte van de mest, kan

*) Hexose Equivalenten; de koolhydraten werden op gewichtsbasis uitgedrukt in equivalente hoeveelheden hexose; zie pagina 16.

tot een foutieve interpretatie leiden, zoals werd ervaren in de zetmeelbehandelingen. Deze maatstaf stemt overeen met de visuele beoordeling, indien de diarree uitsluitend gepaard gaat met een grotere uitscheiding van osmotisch actieve bestanddelen en een daarmee samenhangende toename van de wateruitscheiding. Maar indien ook de excretie toeneemt van bestanddelen die geen osmotische activiteit bezitten (b.v. slijmstoffen) behoeft diarree niet samen te gaan met een lager ds-gehalte in de mest.

Het feit, dat de hoge caseïneverstrekking de mesteigenschappen niet beïnvloedde, leek strijdig te zijn met de resultaten in de literatuur, waar nadrukkelijk werd gewezen op het risico van rottingsdiarree als gevolg van hoge eiwitopname, vooral indien deze gepaard gaat met een verminderde stremming in de lebmaag. Het belang van deze stremming voor de preventie van diarree werd nader onderzocht in Exp. 2 (paragraaf 3.4). Hiertoe werd een kunstmelk rechtstreeks geïnfuseerd in het proximale deel van de duodenum als een kwantitatieve vervanging van de ter plaatse verzamelde chymus. De invloed van deze proefbehandeling op de eigenschappen van de mest werd in de twee opeenvolgende perioden van respectievelijk 4 × 12 uren en 4 × 24 uren gemeten.

Het melkinfuus verhoogde de mestconsistentie aanzienlijk, vooral in de laatste proefperiode (tabel 5). De mest werd zo stevig dat de dieren opvallend veel moeite hadden met de defaecatie. Dit onverwachte, en voorlopige onverklaarbare, resultaat toonde duidelijk aan dat er geen verband bestaat tussen de stremming van melkeiwitten in de lebmaag en rottingsdiarree bij kalveren.

De invloed van onverteerde voerbestanddelen in de chymus van de blinde- en dikke darm op het optreden van diarree werd nader onderzocht in Exp. 3, 4 en 5. Hiervoor werden vijftien kalveren gebruikt met een re-entrant fistel in het distale eind van het ileum (paragraaf 3.5). Het verhoogde aanbod van voederbestanddelen in het achterste gedeelte van het darmkanaal werd gesimuleerd door caseïne, vet of lactose in de distale ileumcannule te infuseren. De dosering van caseïne bedroeg 5 en 10% van het opgenomen re, de vetinfusie 5% van de orale rvet-opname en de lactose-infusie 5, 10 en 20% van de overige koolhydraten (ok)-opname met de controle kunstmelk. Tijdens de proefperioden werden de mestconsistentie (visuele beoordeling), de pH en het ds-gehalte gemeten, evenals de schijnbare verteerbaarheid van de voederbestanddelen (inclusief die van enkele macro-elementen) aan het einde van de dunne darm en in de mest.

De geïnfuseerde voedermiddelen beïnvloedden de mesteigenschappen min of meer identiek als werd waargenomen in Exp. 1 (tabel 6). De dieren reageerden echter betrekkelijk mild op de proefbehandelingen. De caseïne- en vetinfusies hadden geen duidelijk effect op de mest. Slechts de hoogste dosering lactose, 20% van de orale ok-opname, verlaagde alle mestparameters.

Het controlevoer bleek uitstekend verteerbaar in deze proeven (tabel 7). De vertering en resorptie van de meeste bestanddelen was bijna volledig gerealiseerd aan het einde van de dunne darm. Slechts een gering gedeelte van de N en ok verdween in de blinde- en dikke darm en een aanzienlijk deel van het Na. De schijnbare totale verteringscoëfficiënt (vc) van de afzonderlijke voederbestanddelen werden niet of nauwelijks beïnvloed door de caseïne- of vetinfusies (tabel

8). Alleen wanneer de lactosetoediening tot boven 5% van de orale opname steeg, nam de ok-uitscheiding in de mest toe samen met de N-uitscheiding. Vanaf dat niveau steeg de N-uitscheiding lineair met 15–16 mg per g extra geïnfuseerde lactose.

De resultaten van Exp. 1 t/m 5 wezen uit dat een verhoogde opname van melkeiwit van normale voedingskwaliteit, i.c. caseïne, geen risico's inhoudt met het oog op het optreden van rottingsdiarree bij jonge kalveren. Een extra dosering vet of koolhydraten kan leiden tot in voedingsdiarree. De resultaten gaven enkele aanwijzingen dat de toelaatbare hoeveelheid vet in kunstmelk in de praktijk eerder gelimiteerd zal worden door technologische eisen, dan door de grenzen die de dieren aan dit voederbestanddeel stellen. Daarom werd besloten het verdere onderzoek te richten op de koolhydraten. Omdat lactose het belangrijkste koolhydraat is in kunstmelk, kreeg deze suiker de meeste aandacht.

De literatuur met betrekking tot de vertering en resorptie van koolhydraten (hoofdstuk 4) wees uit, dat het jonge kalf lactose uitstekend kan verteren en relatief grote hoeveelheden glucose en galactose kan resorberen. De toelaatbare doseringen van deze bestanddelen in kunstmelk zijn dientengevolge hoog. De mogelijkheden om andere koolhydraten te verteren zijn belangrijk minder ontwikkeld of ontbreken volledig bij jonge kalveren, zoals bijvoorbeeld die voor sacharose. Hun opname is daarom sterk beperkt of zelfs niet toelaatbaar. De literatuur is minder duidelijk over de veranderingen die in het verteringskanaal optreden indien de tolerantie wordt overschreden en fermentatieve diarree optreedt. De consequenties van deze verstoring voor de gezondheid van het dier zijn evenmin systematisch onderzocht. De beperkte informatie leidde tot een viertal vragen. Deze hadden respectievelijk betrekking op: (1) de maximaal toelaatbare hoeveelheid lactose in kunstmelk voor jonge kalveren, (2) het effect van een hoge lactose-opname op zijn vertering en resorptie in de dunne darm, (3) het verband tussen de kwalitatieve en kwantitatieve veranderingen in ileumchymus en fermentatieve diarree en (4) het effect van lactosediarree op de water- en mineralenuitscheiding. Deze vragen werden onderzocht in tien proeven (Exp. 6 t/m 15, hoofdstuk 5).

In Exp. 6 en 7 werd de invloed nagegaan van de lactose-opname op de vertering en resorptie van koolhydraten, gemeten aan de veranderingen in het bloedsuikergehalte in de halsader, en de frequentie van diarree bij jonge kalveren. In de eerste proef werden hiervoor dertig kalveren verdeeld over vijf groepen van zes dieren. De individuele veranderingen in eigenschappen van de mest werden geregistreerd en vergeleken met die in de individuele bloedsuikercurve. Drie doseringen van lactose werden getest gedurende twaalf perioden van drie opeenvolgende dagen. Met de drie proefbehandelingen A, B en C werden dagelijks respectievelijk 10, 13,5 en 17 g Hex. Eq. lactose per kg LG verstrekt, waarvoor drie proefvoeders, A, B en C, werden gebruikt. In Exp. 7, waarbij negen kalveren waren betrokken en alleen de proefbehandelingen A en C werden getest, zijn de proefperioden verlengd van drie tot zeven dagen om een eventuele gewenning van de kalveren aan hoge lactosegiften nader te onderzoeken, aan de hand van dezelfde criteria als werden gebruikt in Exp. 6.

De resultaten van deze beide proeven bevestigden dat bij kalveren snel fermentatieve diarree kan optreden, indien de dagelijkse lactose-opname te hoog is. De toelaatbare grens lijkt ongeveer 10 g Hex. Eq. per kg LG en per dag te zijn bij kalveren van vier weken of ouder. De veranderingen in de bloedsuikercurven wezen uit dat de hoeveelheden lactose die werden afgebroken en geresorbeerd toenamen, naarmate meer melksuiker werd opgenomen. Proefbehandeling C resulteerde in significant hogere curven dan behandeling B, die op zijn beurt de curven significant verhoogde in vergelijking tot het controlevoer A (tabel 14 en 16). De uitkomsten toonden tevens aan dat de dieren met de laagste bloedsuikerresponse meer diarree kregen in de behandelingen B en C. Er waren geen aanwijzingen dat de dieren zich aanpasten aan de hogere lactosegiften gedurende de 7-daagse proefperioden. Wel bleken de kalveren in de eerste vier weken na de geboorte zeer snel te wennen aan het toegenomen lactose-aanbod. Geen duidelijk leeftijdseffect kon worden aangetoond tussen vier en twaalf weken leeftijd. Alleen de helling van de bloedsuikercurve werd geleidelijk steiler naarmate de dieren ouder werden.

De invloed van lactose op de verteringsprocessen in de dunne darm werd nader onderzocht in een serie van zes proeven. De passage en osmotische waarde van de chymus waren de belangrijkste facetten die in Exp. 9, 10 en 11 werden gemeten (paragraaf 5.4.1). In Exp. 9 werden zestien kalveren voor dit doel gebruikt, waarvan een gedeelte was voorzien van re-entrant duodenum- of ileumfistels en een viertal dieren niet was gefistuleerd. Het effect van lactose werd gemeten met behulp van de proefbehandelingen A en C, waarmee respectievelijk 10 en 17 g Hex. Eq. lactose per kg LG en per dag werden verstrekt. De passagesnelheid*) van de chymus, N en reducerende stoffen werden aan het begin van de duodenum en aan het einde van het ileum gemeten in de gefistuleerde kalveren. De niet-geopereerde dieren dienden als 'controle groep' om de diarreeverwekkende invloed van de behandelingen vast te stellen in deze proef. De uitkomsten in Exp. 10, 11 en 13, waarin eveneens metingen in het ileum werden verricht, sloten nauw aan bij die van Exp. 9. In die proeven werden dezelfde lactosebehandelingen getest en tevens proefbehandeling D, welke 8 g Hex. Eq. lactose en 3 g Hex. Eq. sacharose verschafte per kg LG en per dag.

De hoge lactosedosering (behandeling C) wekte in alle proeven fermentatieve diarree op, maar bleek geen invloed uit te oefenen op de verblijfsduur van het voer in de lebmaag, noch op de passagesnelheden van chymus, N, of reducerende stoffen in het begin van de duodenum (tabel 19). De verblijfsduur van de voerbestanddelen in de dunne darm werd evenmin beïnvloed door een hogere lactose-opname. Echter de absolute en relatieve hoeveelheden chymus en reducerende stoffen die aan het einde van het ileum werden opgevangen stegen aanzienlijk in behandeling C; de stikstofpassage veranderde niet (tabel 20). Proefbehandeling D, waarin sacharose werd verstrekt, leidde tot een heftiger reactie van de dieren (tabel 21). In vergelijking tot de lactoseverstrekkingen was

*) Gewicht per tijdseenheid in % van orale opname; zie pagina 51.

de chymuspassage aan het einde van het ileum in proefbehandeling D significant verhoogd en leek ook de verblijfsduur van het voer in lebmaag en dunne darm korter.

In Exp. 12 (paragraaf 5.4.2) werd de invloed van lactose op de schijnbare vc van de voederbestanddelen in de dunne darm onderzocht. Naar aanleiding van de interacties, waargenomen in Exp. 1, werd in deze proef tevens aandacht besteed aan de invloed van de voersamenstelling op dit effect. Vijftien kalveren met re-entrant ileumfistels waren voor dit onderzoek beschikbaar. Ze werden verdeeld over drie groepen van vijf dieren. Iedere groep kreeg hetzij voer A, voer B of voer C in 5 proefperioden van vijf dagen. In de eerste en laatste proefperiode werden deze voeders aangeboden volgens het gebruikelijke schema in de respectievelijke proefbehandelingen A, B en C. In de tussenliggende perioden werden met alle drie voeders dagelijks gelijke hoeveelheden lactose verstrekt, en wel 15,3, 12,2 en 9 g Hex. Eq. per kg LG in P_2, P_3 en P_4 successievelijk. De ileumchymus werd kwantitatief opgevangen en geanalyseerd.

Tijdens de proef kwam tot uiting dat de voeropname- en verteringscapaciteit van deze kalveren geleidelijk verminderde naarmate de proef langer duurde. Dit verhinderde een nauwkeurige schatting van de schijnbare verteringscoëfficiënten in de dunne darm. Een covariantie analyse toonde aan dat de schijnbare vc van de diverse voederbestanddelen niet, of slechts in geringe mate werden beïnvloed door de lactose-opname (tabel 22). Slechts de koolhydratenfractie werd relatief minder efficiënt verteerd in behandeling C vergeleken met de behandelingen A en B. Deze hoge dosering leek ook iets nadelig voor de schijnbare vc van het ruweiwit. De uitkomsten bevestigden dat de absolute hoeveelheden lactose, die in de dunne darm worden verteerd en geresorbeerd, toenemen naarmate het lactose-aanbod stijgt. Maar deze toename is onvoldoende om een hogere koolhydraten passage naar de dikke darm te voorkomen. De resultaten toonden verder aan dat de schijnbare vc van de voederbestanddelen nauwelijks en niet systématisch worden beïnvloed door de voersamenstelling. De geringe verschillen die in Exp. 12 werden waargenomen tussen de drie rantsoenen stonden niet in verhouding tot die, welke in Exp. 1 werden gevonden tussen de resultaten na toevoeging van afzonderlijke componenten en van de kunstmelk. Het bevestigde dat deze laatste verschillen hoofdzakelijk een gevolg waren van verschillen in microbiële fermentatie in de blinde- en dikke darm. De maximaal toelaatbare lactosegift is volgens deze uitkomsten een absolute hoeveelheid, afhankelijk van het lichaamsgewicht van de dieren en niet afhankelijk van het lactosegehalte in het voer.

In de twee hierop volgende proeven, Exp. 13 en 14, werd onderzocht of lactase, dan wel de hexose-absorptie in de dunne darm limiterend is voor de hoeveelheid lactose die maximaal toegelaten kan worden in voeders voor jonge kalveren. De voorgaande proeven gaven hierover onvoldoende uitsluitsel, vooral omdat er in ileumchymus en mest een discrepantie bestond tussen de uitkomsten van de analyses op reducerend vermogen en de enzymatische bepaling van de individuele suikers, glucose, galactose en lactose. Exp. 13 was opgezet om dit verschil nader te onderzoeken en tevens om meer inzicht te krijgen in de gehalten van de

afzonderlijke suikers in ileumchymus en mest. Hiertoe werden voer A en C verstrekt aan vijf kalveren, voorzien van re-entrant ileumfistels. De chymus en mest werden in afzonderlijke proefperioden kwantitatief opgevangen. De monsters werden geanalyseerd op reducerende stoffen en op glucose, galactose en lactose, bepaald volgens twee verschillende enzymatische analyse methoden.

De resultaten (tabel 24, 25 en 26) toonden aan dat glucose bijna volledig wordt verteerd in de dunne darm. In overeenstemming met de literatuur is de galactose resorptie in de dunne darm vertraagd, vooral indien het lactose-aanbod toeneemt. De hoeveelheden lactose die onverteerd de dunne darm verlaten zijn klein in vergelijking met de totale hoeveelheid galactose. Een belangrijk gedeelte van de galactose was in deze proef gebonden in één of meer andere oligosachariden. De oligosachariden bevatten, gezamenlijk, ongeveer 4,5 à 4,6 maal zoveel galactose als glucose moleculen. Het feit, dat deze stoffen niet werden aangetroffen in de melk of in duodenumchymus, gaf een sterke aanwijzing dat ze werden gesynthetiseerd tijdens het verteringsproces van lactose.

Dit laatste aspect, de conversie van lactose door lactase tot andere oligosachariden in de dunne darm van kalveren, werd nader onderzocht in Exp. 14. In deze proef werd het „lactose" rantsoen C of een „hexose" (glucose + galactose) rantsoen C′ verstrekt. Met beide behandelingen werd dagelijks 16 à 17 g Hex. Eq. per kg LG verstrekt aan tien kalveren, voorzien van re-entrant ileumfistels, en aan acht niet-gefistuleerde kalveren. De ileumchymus en de mest van de gefistuleerde dieren werden kwantitatief verzameld en gaschromatografisch onderzocht op de gehalten van de afzonderlijke koolhydraten. De overige dieren fungeerden als „controle groep" om meer exact de mestresponse te meten.

Beide kunstmelken veroorzaakten fermentatieve diarree (tabel 27), waarbij behandeling C′ gevaarlijker bleek te zijn. Die behandeling resulteerde in een opvallende teruggang in conditie van de dieren en veroorzaakte tevens beschadigingen in de darmmucosa. Na drie weken kon nog steeds een nawerking van deze proefbehandeling worden onderkend. Het verschil tussen beide voeders was ook af te lezen aan de passage van de ileumchymus, die in behandeling C′ hoger was dan op het lactoserantsoen C (tabel 28).

De suikeranalyses wezen uit dat in de lactosebehandeling een belangrijk deel van de hexosen in de ileumchymus gebonden waren in lactose, maltose en in een aantal andere di- en trisachariden, waarvan galactose het hoofdbestanddeel vormde. De monsters, verzameld in behandeling C′, bevatten meer (totaal) glucose en galactose dan in de lactosebehandeling. In deze behandeling werden echter hoofdzakelijk vrije hexosen aangetoond; het aandeel van de oligosachariden was bijna te verwaarlozen. In de mest werden hoofdzakelijk vrije glucose en galactose aangetroffen (tabel 29). Sporen lactose en andere disachariden kwamen uitsluitend voor in proefbehandeling C. Trisachariden werden niet aangetroffen in de mest. De totale glucose en galactose uitscheiding in de mest was, hoewel gering in beide behandelingen, iets hoger ingeval lactose werd gevoerd.

De uitkomsten van Exp. 13 en 14 wijzen uit dat in de dunne darm van kalveren conversie van lactose optreedt onder invloed van lactase. Hierbij ontstaan diverse oligosachariden die hoofdzakelijk uit galactose bestaan. Dit verschijnsel ver-

klaart echter niet de geconstateerde verschillen in glucose- en galactoseresorptie in de dunne darm; de schijnbare vc van galactose in de dunne darm was hoger indien lactose werd gevoerd, dan wanneer de hexosen glucose en galactose werden aangeboden. Tevens bevestigen deze uitkomsten zeer duidelijk dat niet de lactase, maar de absorptie van galactose de toelaatbare grens van lactose vaststelt bij jonge kalveren. Hoewel in onze proeven enige lactose werd aangetoond aan het einde van de dunne darm, was deze hoeveelheid klein in verhouding tot de totale hoeveelheid galactose ter plaatse. Daarbij is het niet uitgesloten dat deze lactose deels, of volledig een eindprodukt was van de transgalactosylering door lactase. De verhouding tussen de concentraties en de verschillen in osmotische activiteit van de diverse koolhydraten in de ileumchymus tonen aan dat de hoeveelheid galactose primair verantwoordelijk is voor de veranderingen in de ileumpassage bij hoge lactosegiften.

Het verband tussen de kwantitatieve en kwalitatieve veranderingen in ileumchymus en fermentatieve diarree werden in twee proeven getoetst (Exp. 8 en 11, paragraaf 5.5). In de laatste proef werd de ileumchymus kwantitatief opgevangen bij kalveren, die de sacharosebehandeling D ontvingen en „gekruist" geïnfuseerd in partner-kalveren, die het controlevoer A kregen, en omgekeerd. Als gevolg hiervan werd de nadelige invloed van rantsoen D op de mestconsistentie eveneens „gekruist" waargenomen. De dieren op voer A kregen ernstige voedingsdiarree, terwijl de mest van de andere groep normaal bleef, ondanks de opname van rantsoen D (tabel 30).

Hoewel de toename van de koolhydraten in de chymus in de literatuur verantwoordelijk wordt gesteld voor de diarree-verwekkende eigenschappen van ileumchymus, was de reactie van de dieren in Exp. 5, waar lactose aan de chymus werd toegevoegd, zeer mild. Daarom werd besloten deze proef te herhalen (Exp. 8). Naar aanleiding van de ervaringen bij kalveren die sterk reageerden op behandeling C werden galactose en lactose in het distale eind van het ileum geïnfuseerd in een dosering van 40% van de orale opname. De suikers werden geïnfuseerd in een waterige oplossing, isotonisch met de ileumchymus (312 m osmol/L). Een isotonische zoutoplossing diende in deze proef als controle. De toediening van de koolhydraten aan ileumchymus verminderde snel de mestconsistentie, pH en het ds-gehalte (tabel 31). De reactie was nagenoeg identiek aan die, waargenomen bij de sterk reagerende dieren op behandeling C in de andere proeven. Ze bevestigden de zienswijze dat de koolhydraten in ileumchymus primair verantwoordelijk zijn voor de diarreeverschijnselen.

De invloed van de fermentatieve diarree op de wateruitscheiding werd in een aantal proeven nagegaan, waarbij de urine-excretie en de wateruitscheiding met de mest in de proefbehandelingen A, B en C kwantitatief werden gemeten (paragraaf 5.6). De uitkomsten suggereerden dat fermentatieve diarree niet tot een groter waterverlies hoeft te leiden en zeker niet tot uitdrogingsverschijnselen, zoals die bij andere vormen van diarree zijn waargenomen. De verhoogde wateruitscheiding met de mest tijdens fermentatieve diarree, wordt volledig gecompenseerd door een lagere urine-excretie (tabel 32). De koolhydraten uitscheiding met urine, en in het bijzonder die van galactose, neemt echter toe naarmate de

lactose-opname stijgt (tabel 33).

In de laatste proef is aandacht geschonken aan de invloed van hoge lactosegiften op de mineralenresorptie (Exp. 15). De proefbehandelingen A, B en C werden verstrekt aan twintig kalveren, waarvan tien waren voorzien van een re-entrant ileumfistel. De resorptie van Na, K, Cl, Ca, P en Mg in de dunne darm werd bij de gefistuleerde dieren onderzocht. De overige kalveren dienden om de totale resorptie en de retentie van deze elementen te meten.

Het controlevoer A resulteerde, in overeenstemming met de literatuur en de uitkomsten in Exp. 3 en 5, in vrij hoge schijnbare resorptiecoëfficiënten van de mineralen in de dunne darm (tabel 34). Het merendeel van de mineralen werd reeds geresorbeerd in de dunne darm. In de dikke darm werd uitsluitend een aanzienlijke resorptie van Na vastgesteld. De verhoogde lactosegiften resulteerden in ernstige diarree en verlaagden de resorptie van K, Cl, Ca en vooral die van Na in de dunne darm. Hetzelfde effect werd waargenomen bij de totale resorptie en de retentie van deze mineralen. De invloed van lactose op deze parameters was echter minder groot en alleen significant voor Na en K (tabel 35). Bij de meeste dieren werd een positieve mineralenbalans waargenomen, ondanks de ernst van de diarree in deze proef. Niettemin werd bij enkele individuele kalveren een netto verlies van Na vastgesteld (tabel 36).

Uit de vijftien beschreven proeven is af te leiden, dat melksuiker uitstekend wordt verteerd en geresorbeerd door jonge kalveren en als enige disacharide in grote hoeveelheden in kunstmelk voor kalveren kan worden opgenomen. De maximale hoeveelheid lactose die door deze dieren wordt getolereerd lijkt zelfs iets groter te zijn dan die van glucose en galactose als gevolg van een lagere osmotische activiteit van lactose en zijn conversie produkten in het darmkanaal en de hogere resorptie in de dunne darm. In hoeverre de oligosachariden, onstaan tijdens de vertering van lactose, hierbij daadwerkelijk een gunstige invloed uitoefenen is nog niet exact vastgesteld. Indien de lactose-opname hoger wordt dan de limiet die door de galactoseresorptie wordt gesteld (ongeveer 10 g Hex. Eq. lactose per kg LG en per dag), treedt snel fermentatieve diarree op. De daarmee gepaard gaande veranderingen in darmchymus en eigenschappen van de mest verschillen niet principieel van die, welke worden waargenomen bij mensen die lijden aan hypolactasia.

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APPENDIX

EXPERIMENT 1.

EFFECTS OF THE COMPOSITION AND INTAKE OF MILK SUBSTITUTE DIETS ON FAECAL CHARACTERISTICS.

Number of calves: 6.
Age at start: 5 weeks.
Experimental periods: 12 × 3 days; 4 days recovery between each period.

Experimental design[1]*:*

Exp. periods	Treatments	Additional supply as % of control diet					
		Calf 1	Calf 2	Calf 3	Calf 4	Calf 5	Calf 6
P_1	–	–	–	–	–	–	–
P_2	lactose	75	75	–	37.5	–	37.5
P_3	control diet	37.5	75	37.5	–	–	75
P_4	lactose	37.5	37.5	125	87.5	125	87.5
P_5	sucrose	37.5	37.5	75	–	75	–
P_6	lactose	37.5	37.5	125	87.5	125	87.5
P_7	gelatinized corn starch	37.5	–	37.5	75	75	–
P_8	raw corn starch	12.5	–	12.5	30	30	–
P_9	lactose	37.5	37.5	125	87.5	125	87.5
P_{10}	casein	70	70	140	140	–	–
P_{11}	fats	35	60	35	–	–	60
P_{12}	lactose	37.5	37.5	125	87.5	125	87.5

[1] The control diet was fed at a daily rate of 17 g milk replacer per kg BW, supplying 8 g Hex. Eq. per kg BW. In several experimental periods additional amounts of carbohydrates were supplied, increasing the Hex. Eq. supply as indicated in the design. In P_3 the control diet supply was increased by 37.5 and 75%, respectively. In P_{10}, P_{11} the percentages represent the increase in dietary CP and EE, respectively.

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Faecal characteristics
- visual score,
- pH,
- DM content.

Diet composition:

	Control diet	P_{10} 'High' casein-diets (%)		P_{11} 'High' fat-diets (%)		
		Relative protein increase:		Relative fat increase:		
	(%)	70%	140%	0%	35%	60%
skim milk powder[1]	60.3	45.0	29.5	66.7	65.3	54.9
de-lactosed whey powder[1]	7.0	5.2	3.5	–	–	–
whey powder[1]	8.6	6.5	4.3	–	–	–
Na-caseinate	–	15.5	31.0	–	–	–
casein	–	9.5	19.0	–	–	4.0
fats[2]	19.0	14.2	9.5	19.8	29.7	39.8
gelatinized corn starch	2.2	1.6	1.1	–	–	–
dextrose	1.7	1.3	0.9	6.0	–	–
premix[3]	1.2	1.2	1.2	1.2	1.3	1.3
Chemical composition:						
DM %	97.2	97.4	97.4	97.3	97.2	97.3
CP (N × 6.38) %	25.0	42.0	60.0	23.9	23.4	24.0
EE %	19.0	14.0	9.5	19.8	29.7	39.8
NFE %	45.9	34.4	22.9	47.8	38.3	29.0
ME (MJ/kg)	18.3	17.8	17.3	18.8	20.9	23.1

[1] All milk and whey powders used were spray-dried.

[2] Consisted of: 68% animal fat, 25% vegetable fat, 7% emulsifier (glycerin-polyglycol-fatty ester + lecithin).

[3] Composition per kg premix:
250 g $CaHPO_4$; 125 g $CaCl_2.2\ H_2O$; 83.3 g NaCl; 83.3 g MgO; 3.7 g ZnO; 34 mg KJ; 10.4 mg $Na_2SeO_3.5H_2O$ and 13.8 St.U. vit. A; 2.8 St. U. vit. D_3; 1.42 d-α-tocopherol; 4.26 mg menadione; 425 mg thiamine; 710 mg riboflavin; 1.67 mg vitamin B_{12}; 283 mg pyridoxine; 1775 mg Ca-pantothenate; 3.84 g niacin; 28.4 g choline chloride; 5.7 g vit. C and 6.2 g penicillin-streptomycin; 4.2 g furazolidone.

20 ppm Fe was added to all diets to prevent anaemia. The diets were diluted with water in a ratio of 1 : 5.5.

Frequency of feeding: Twice daily; 8^{00} and 16^{00} h.

Miscellaneous: To ensure equal ME intakes in P_{11} the daily allowances of the 3 diets were: 17, 15.3 and 13.6 g per kg BW, providing 3.34, 4.54 and 5.41 g fat, respectively.

EXPERIMENT 2.

THE EFFECTS OF ABOMASAL CLOTTING ON FAECAL CHARACTERISTICS.

Number of calves: 2; fitted with re-entrant duodenal cannulae.
Age at start: 7 weeks.
Experimental periods: 2 × 4 days; 3 days recovery between each period.

Experimental design:

Exp. period	Diet	Duodenal infusion
P_1	A	4 days; from 8^{00}–20^{00} h.
P_2	A	4 × 24 h.

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Duodenal digesta flow – flow rate of wet digesta,
– DM, ash, EE and N content.
c. Faecal characteristics – visual score,
– pH.

Diet composition[1].		Diet A (%)
skim milk powder		65.6
lactose		15.4
fats		16.8
lecithin		1.0
premix		1.2
Fe premix[2]		0.1
Chemical composition:		
DM	%	96.4
CP (N × 6.38)	%	22.9
EE	%	18.0
NFE	%	48.4
ME	MJ/kg	18.6

[1] See Exp. 1 for detailed information.
[2] Contained 2% Fe.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: Diet A was fed at a daily rate of 17.7 g per kg BW. Abomasal chyme was collected quantitatively and replaced by milk A, using the 'sampling apparatus'. The dilution rate of the infusate compensated for the difference in weight between oral intake and abomasal outflow. This difference was estimated to be 70% of oral weight intake. That quantity was additionally infused with a 0.4% NaCl solution.

EXPERIMENT 3.

THE EFFECT OF CASEIN AND FAT INFUSIONS INTO THE LOWER INTESTINE ON APPARENT DIGESTIBILITIES AND FAECAL CHARACTERISTICS.

Number of calves: 6; fitted with re-entrant ileal cannulae.
Age at start: 7–8 weeks.
Experimental periods: 5 × 5 days; 2 days recovery between each period.

Experimental design:

Exp. period	Infusate[1]	Sampled parameter
P_1	–	faeces
P_2	–	ileal digesta
P_3	5% casein	faeces
P_4	10% casein	faeces
P_5	5% fat	faeces

[1] The amounts infused are expressed as % of oral N or EE intake.

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Ileal digesta flow rate.
c. Faecal characteristics
   - visual score,
   - pH,
   - DM content.
d. Samples were analysed for: DM, ash, N, EE, Na, K, Ca and P.

Diet composition[1]:		Commercial milk replacer (%)
skim milk powder		68.2
lactose		6.8
dextrose		5.9
fats		17.8
premix		1.2
Fe premix		0.1
Chemical composition:		
DM	%	97.2
CP (N × 6.38)	%	25.4
EE	%	17.8
NFE	%	47.2
ME	MJ/kg	18.1
Na	%	0.36
K	%	1.07
Ca	%	0.98
P	%	0.72

[1] For detailed information see Exp. 2.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: The control diet was fed in amounts based on the standard feeding schedule. To avoid refusals, the daily allowance was reduced by 10%. In P_2 the apparent digestibility of the organic feed components and the absorption rate of the cations were measured in the small intestine. In the other experimental periods similar measurements were made on the faeces. The infusates were mixed with ileal chyme collected beforehand. This mixture was returned at body temperature to the calves through the re-entrant cannula.

EXPERIMENT 4.

THE EFFECT OF CASEIN INFUSIONS INTO THE LOWER INTESTINE ON APPARENT DIGESTIBILITIES AND FAECAL CHARACTERISTICS.

Number of calves: 6; fitted with re-entrant ileal cannulae.
Age at start: 4 weeks.
Experimental periods: 5 × 5 days, 2 days recovery between each period.

Experimental design:

Exp. period	Infusate[1]	Sampled parameter
P_1	–	faeces
P_2	–	ileal digesta
P_3	5% casein	faeces
P_4	10% casein	faeces
P_5	–	ileal digesta

[1] See Exp. 3. Blockage of the cannulae by the infusate prevented testing 20% casein, as was intended between P_4 and P_5.

Measurements and analyses: See Exp. 3. The electrolytes were not analysed.

Diet composition[1]:		Commercial milk replacer (%)
skim milk powder		69.0
lactose		6.0
dextrose		5.7
fats		18.0
premix		1.2
Fe premix		0.1
Chemical composition:		
DM	%	97.1
CP (N × 6.38)	%	25.6
EE	%	19.8
NFE	%	45.4
ME	MJ/kg	18.7

[1] For detailed information see Exp. 2.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: See Exp. 3. The infusion procedure was changed. Casein was dissolved in water and continuously infused into the distal ileal cannulae.

EXPERIMENT 5.

THE EFFECT OF LACTOSE INFUSIONS INTO THE LOWER INTESTINE ON APPARENT DIGESTIBILITIES AND FAECAL CHARACTERISTICS.

Number of calves: 3; fitted with re-entrant ileal cannulae.
Age at start: 4 weeks.
Experimental periods: 5 × 5 days; 2 days recovery between each period.

Experimental design:

Exp. period	Lactose infusions to calf:[1]			Sampled parameter
	1	2	3	
P_1	–	–	–	faeces
P_2	–	–	–	ileal digesta
P_3	5%	20%	10%	faeces
P_4	20%	10%	5%	faeces
P_5	–	–	–	ileal digesta

[1] Lactose infusate expressed as % of oral NFE intake.

Measurements and analyses: See Exp. 3. Only the electrolytes Na and K were analysed.

Diet composition[1]*:*		Commercial milk replacer (%)
skim milk powder		69.7
whey powder		6.0
dextrose		4.0
fats		19.0
premix		1.2
Fe premix		0.1
Chemical composition:		
DM	%	97.2
CP (N × 6.38)	%	25.2
EE	%	19.5
NFE	%	45.6
ME	MJ/kg	18.5
Na	%	0.50
K	%	1.66

[1] For detailed information see Exp. 2.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: See Exp. 3. An aqueous solution of lactose was gradually infused into the distal ileal cannula.

EXPERIMENT 6.

THE EFFECT OF VARYING LACTOSE INTAKES ON BLOOD SUGAR LEVELS IN V. JUGULARIS ON FAECAL CHARACTERISTICS.

Number of calves: 30 (5 × 6).
Age at start: 1 week.
Experimental periods: 12 × 3 days; 4 days recovery between each period.
Experimental design: Three treatments were tested:
A. 19.65 g diet A per kg BW per day (= 10 g Hex. Eq.),
B. 21.95 g diet B per kg BW per day (= 13.5 g Hex. Eq.),
C. 23.70 g diet C per kg BW per day (= 17 g Hex. Eq.).

Exp. period	Control groups[1]		Experimental groups		
	1^A	1^B	2	3	4
P_1	A	A	A	B	C
P_2	A(C)	A	B	C	A
P_3	A	A	C	A	B
P_4	A(C)	A	B	A	C
P_5	A	A	A	C	B
P_6	A(C)	A	C	B	A
P_7	A	A	A	B	C
P_8	A(C)	A	B	C	A
P_9	A	A	C	A	B
P_{10}	A(C)	A	B	A	C
P_{11}	A	A	A	C	B
P_{12}	A(C)	A	C	B	A

[1] The control groups served to check (quantitatively) a possible carry-over effect of the treatments on the calves responses. Group 1^B was designed to control the responses in treatment A and group 1^A, receiving treatment C only at one feeding time in a fortnight, served to check the response in that treatment.

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Faecal characteristics
- visual score,
- pH,
- DM content.

c. Blood sugar level in V. jugularis, except in P_5, P_7 and P_9.

Diet composition[1]:		Diet A (%)	Diet B (%)	Diet C (%)
skim milk powder		68.6	61.2	56.8
lactose		12.5	28.9	39.1
fats		16.7	7.9	2.25
lecithin		1.0	0.9	0.84
premix		1.2	1.07	1.00
Fe premix		0.15	0.135	0.125
Chemical composition:				
DM	%	97.5	97.6	97.6
CP (N × 6.38)	%	25.1	23.2	21.4
EE	%	17.9	9.0	3.2
NFE	%	48.3	59.7	67.8
ME	MJ/kg	18.3	16.5	15.3

[1] For detailed information see Exp. 2. The composition of the three diets was based on the intended intakes of Hex. Eq. at equal intakes of CP and ME. Diet dilution rate was based on equal water intakes in the three treatments.

Feeding frequency: Twice daily; 8^{00} and 16^{00} h.

Miscellaneous: Blood samples were taken manually from each calf before and after the first feeding time in the Exp. periods. Blood was sampled $\frac{1}{2}$ h pre-feeding and at $\frac{1}{2}$, $1\frac{1}{2}$, 2, 3, $4\frac{1}{2}$, 6 and $7\frac{1}{2}$h post -feeding.

In P_1, P_2 and P_3 the feeding level was reduced to 55, 80 and 90% of the intended one, because of the low intakes of calves in their first weeks of life.

EXPERIMENT 7.

ADAPTATION TO HIGH LACTOSE INTAKES.

Number of calves: 10 (2 × 5); fitted with permanent V.jugularis cannulae.
Age at start: 5 weeks.
Experimental periods: 2 × 7 days; 7 days recovery between each period.

Experimental design:

Exp. period	Group 1	Group 2
P_1	A	C
P_2	C	A

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Faecal characteristics – visual score,
– pH.
c. Blood sugar levels on day 1, 4 and 7 in each period.

Diet composition[1]*:*		Diet A (%)	Diet C (%)
skim milk powder		66.7	55.4
lactose		13.9	39.9
fats		17.1	2.7
lecithin		1.0	0.9
premix		1.2	1.0
Fe premix		0.1	0.1
Chemical composition:			
DM	%	97.2	97.3
CP (N × 6.38)	%	25.0	21.0
EE	%	17.3	3.3
NFE	%	49.0	68.0
ME	MJ/kg	18.2	15.3

[1] For detailed information see Exp. 6. Penicillin-streptomycin was replaced by 7.5 g Zn-bacitracin per kg premix.

Feeding frequency: Twice daily; 8^{00} and 17^{30} h.

Miscellaneous: Blood sampling times were slightly changed compared with Exp. 6; $\frac{1}{2}$ h before and $\frac{1}{2}$, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, $4\frac{1}{2}$, 6 and 9 h post-feeding.

EXPERIMENT 8.

THE EFFECT OF LACTOSE AND GALACTOSE INFUSIONS INTO THE LOWER INTESTINE ON FAECAL CHARACTERISTICS.

Number of calves: 12 (3 × 4); fitted with ileal cannulae.
Age at start: 5 weeks.
Experimental periods: 3 × 3 days; 4 days recovery between each period.

Experimental design:

Exp. period	Treatments[1]		
	Group I	Group II	Group III
P_1	1	2	3
P_2	4	4	4
P_3	3	1	2

[1] compound infused: quantity infused (% of oral intake):
1. lactose 40% of NFE.
2. galactose 40% of NFE.
3. galactose 40% of reducing substances (17.5% of NFE).
4. NaCl solution the amount of water was equal to that used in treatment 1.

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Faecal characteristics
- visual score,
- pH,
- DM content.

c. DM, ash, N, and EE were analysed in all samples.
d. Faecal osmolality.

Diet composition[1]*:*		Diet A (%)
skim milk powder		66.3
lactose		15.0
fats		16.4
lecithin		1.0
premix		1.2
Fe premix		0.1
Chemical composition:		
DM	%	96.6
CP (N × 6.38)	%	24.2
EE	%	18.6
NFE	%	47.6
ME	MJ/kg	18.3

[1] For detailed information see Exp. 7.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: All components were infused in an aqueous solution, isotonic with ileal digesta. The solutions were continuously pumped into the distal ileal cannulae.

EXPERIMENT 9.

THE EFFECTS OF HIGH LACTOSE INTAKE ON DUODENAL AND ILEAL DIGESTA FLOW RATES.

Number of calves: 16 (8 × 2); 8 animals fitted with duodenal re-entrant cannulae, 4 with re-entrant ileal cannulae, and 4 non-fistulated.
Age at start: 4 weeks.
Experimental periods: 4 × 3 days; 4 days recovery between each period, except between P_2 and P_3 when 11 days recovery was allowed.
Experimental design: For detailed information about treatments A and C see Exp. 6.

Exp. period	Non-fistulated		Ileal-fistulated		Duodenal-fistulated[1]	
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
P_1	A	C	A	C	A	C
P_2	C	A	C	A	C	A
P_3	A	C	A	C	A	C
P_4	C	A	C	A	C	A

[1] The 4 animals used in P_1, P_2 were replaced by 4 others in P_3, P_4 to avoid the risk of leakage around the cannulae.

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Faecal characteristics in group 1 and 2 – visual score, – pH, – DM content.
c. Urine excretion in group 1 and 2 – weight, – reducing substances content.
d. Duodenal digesta flow rate – weight of wet digesta, – N and reducing substances content.
e. Ileal digesta flow rate – weight of wet digesta, – N and reducing substances content.

Diet composition[1]:		Diet A (%)	Diet C (%)
skim milk powder		68.6	56.8
lactose		12.5	39.1
fats		16.7	2.25
lecithin		1.0	0.84
premix		1.2	1.0
Fe premix		0.1	0.1
Chemical composition:			
DM	%	96.8	97.1
CP (N × 6.38)	%	24.6	20.3
EE	%	18.1	3.4
NFE	%	48.3	68.9
ME	MJ/kg	18.3	15.3

[1] For detailed information see Exp. 7.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: Faeces and urine were quantitatively collected during 3 × 24 h in each period. Duodenal and ileal digesta were collected from 8^{00}–20^{00} h.

EXPERIMENT 10.

THE EFFECT OF HIGH SUCROSE INTAKE ON FLOW RATE OF ILEAL DIGESTA.

Number of calves: 4; 2 of which fitted with re-entrant ileal cannulae.
Age at start: 4 weeks.
Experimental periods: 3 × 3 days; 4 days recovery between each period.
Experimental design:

Exp. period	Non-fistulated	Fistulated
P_1	A[1]	A
P_2	D[1]	D
P_3	A	A

[1] Treatment A: 17.7 g diet A per kg BW per day, providing 9 g Hex. Eq. lactose per kg BW.
Treatment D: 19.5 g diet D per kg BW per day, providing 9 g Hex. Eq. lactose and 2.7 g Hex. Eq. sucrose per kg BW.

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Faecal consistency (in the non-fistulated calves) – visual score,
– pH.
c. Urine excretion (in the non-fistulated calves) – weight,
– reducing substances content.
d. Ileal flow rate – wet digesta weight,
– wet digesta pH,
– wet digesta osmolality.

Diet composition[1]:		Diet A (%)	Diet D (%)
skim milk powder		66.9	61.2
lactose		15.4	13.2
sucrose		–	13.2
fats		15.5	9.8
lecithin		1.0	0.6
premix		1.2	1.1
Fe premix		0.1	0.1
Chemical composition:			
DM	%	97.1	97.2
CP (N × 6.38)	%	24.5	22.7
EE	%	18.1	11.9
NFE	%	48.4	57.2
ME	MJ/kg	18.0	⩾ 15.0

[1] For detailed information see Exp. 7.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: See Exp. 9.

EXPERIMENT 11.

THE EFFECT OF HIGH LACTOSE AND SUCROSE INTAKES ON ILEAL DIGESTA FLOW RATES. THE DIARRHOEIC PROPERTIES OF ILEAL DIGESTA.

Number of calves: 8 (2 × 4); fitted with re-entrant ileal cannulae. In P_5 one group of 6 animals was selected and in P_6 2 groups each of 3 animals.

Age at start: 5 weeks.

Experimental periods: 4 × 4 days; 3 days recovery between each period (P_1–P_4),
1 × 5 days; 2 days recovery (P_5),
1 × 2 days (P_6).

Experimental design:

Exp. period	Treatments[1]	
	Group I	Group II
P_1	A	C
P_2	C	A
P_3	A	C
P_4	C	A
P_5	D	
P_6[2]	A	D

[1] Treatments A and C: see Exp. 6.
Treatment D: 18.7 g diet D per kg BW per day, providing 8 g Hex. Eq. lactose and 3 g Hex. Eq. sucrose per kg BW.

[2] Ileal digesta collected in group I, receiving diet A, was infused in counter-part calves of group II receiving diet D, and vice versa.

Measurements and analyses:

a. Diet composition and daily feed intake.

b. Faecal characteristics in P_5 (day 1 and 2) and P_6:
- visual score,
- pH.

c. Urine excretion in P_5 (day 1 and 2) and P_6:
- weight,
- reducing substances content.

d. Ileal digesta flow rate:
- weight (P_1–P_6; in P_5 at day 3 and 4),
- pH, osmolality, reducing substances, lactose, free glucose, free galactose (in P_1–P_4),
- FFA in aliquot samples (in P_1–P_4).

Diet composition[1]:		Diet A (%)	Diet C (%)	Diet D (%)
skim milk powder		66.8	55.5	56.1
lactose		13.8	39.9	11.6
sucrose		–	–	16.1
fats		17.2	2.8	14.4
lecithin		1.0	0.84	0.84
premix		1.2	1.0	1.0
Fe premix		0.1	0.08	0.08
Chemical composition:				
DM	%	97.6	97.6	97.7
CP (N × 6.38)	%	25.0	20.9	21.0
EE	%	17.8	3.5	14.9
NFE	%	48.4	68.2	48.4
ME	MJ/kg	18.3	15.4	⩾15.3

[1] For detailed information see Exp. 7.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: See Exp. 9. Ileal digesta was collected from 8^{00}–20^{00} h except in P_6, where the collections lasted 2 × 24 h.

EXPERIMENT 12.

EFFECTS OF DIET COMPOSITION AND LEVEL OF INTAKE ON THE APPARENT DIGESTIBILITY OF ITS COMPONENTS IN THE SMALL INTESTINE.

Number of calves: 15 (3 × 5); fitted with re-entrant ileal cannulae.
Age at start: 5 weeks.
Experiment periods: 5 × 5 days; 2 days recovery between each period.
Experimental design:

Exp. period	Daily intake (g Hex. Eq. per kg BW)		
	Group I Diet A	Group II Diet B	Group III Diet C
P_1	9	12.2	15.3
P_2	12.2	12.2	12.2
P_3	15.3[1]	15.3	15.3
P_4	9	9	9
P_5	9	12.2	15.3

[1] In Group I this level was reduced to 12.2 g because of the refusals expected to occur.

Measurements and analyses:
a. Diet composition and feed intake.
b. Ileal digesta flow rate – weight of wet digesta.
c. Samples were analysed on – DM, ash, N, EE, reducing substances, free glucose, free galactose and lactose.

Diet composition[1]*:*		Diet A (%)	Diet B (%)	Diet C (%)
skim milk powder		66.6	61.0	55.4
lactose		13.9	26.9	39.9
fats + lecithin		18.2	10.9	3.6
premix		1.2	1.1	1.0
Fe premix		0.1	0.09	0.08
Chemical composition:				
DM	%	97.2	97.3	97.6
CP (N × 6.38)	%	24.1	22.0	19.7
EE	%	18.2	11.0	3.9
NFE	%	49.2	59.1	69.2
ME	MJ/kg	18.4	16.9	15.4

[1] For detailed information see Exp. 7.

Feeding frequency: Twice daily 8^{00} and 20^{00} h.

Miscellaneous: Ileal chyme was quantitatively collected for 5 × 24 h in the periods. The samples were each day combined per group for analysis.

EXPERIMENT 13.

THE CARBOHYDRATE COMPOSITION OF ILEAL DIGESTA AND FAECES.

Number of calves: 5; fitted with re-entrant ileal cannulae.
Age at start: 5 weeks.
Experimental periods: 4 × 4 days; 3 days recovery between each period.
Experimental design:

Exp. period	Treatment[1]	Sampled parameter
P_1	A	faeces
P_2	A	ileal digesta
P_3	C	faeces
P_4	C	ileal digesta

[1] For detailed information see Exp. 6.

Measurements and analyses:
a. Diet composition and daily feed intake.
b. Faecal characteristics – visual score,
– pH,
– weight.
c. Ileal flow rates – weight of wet digesta.
d. Samples were analysed on – reducing substances, free glucose, free galactose, di- and oligosaccharides.

Diet composition[1]*:*		Diet A (%)	Diet C (%)
skim milk powder		66.6	55.9
lactose		13.6	39.3
fats + lecithin		18.5	3.7
premix		1.2	1.0
Fe premix		0.1	0.08
Chemical composition:			
DM	%	96.8	97.5
CP (N × 6.38)	%	24.2	20.5
EE	%	18.1	3.9
NFE	%	48.6	68.0
ME	MJ/kg	18.3	15.4

[1] For detailed information see Exp. 7.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: Ileal digesta were collected from 8^{00}–20^{00} h and the samples from two successive hours were in each treatment combined for analysis. Aliquot samples from faeces collected from 8^{00}–20^{00} h and from 20^{00}–8^{00} h from each treatment were pooled for analysis.

Carbohydrate analysis was carried out according to method I and II.

EXPERIMENT 14.

THE EFFECT OF HIGH INTAKES OF LACTOSE OR HYDROLYSED LACTOSE ON FLOW RATES AND COMPOSITION OF ILEAL DIGESTA AND FAECAL CHARACTERISTICS.

Number of calves: 18 (10 + 8); 10 fitted with re-entrant ileal cannulae.
Age at start: 5 weeks.
Experimental periods: 5 × 4 days; 6 days recovery between P_2 and P_3; 13 days recovery between P_4 and P_5.
Experimental design:

Exp. period	Fistulated calves		Non-fistulated calves	
	Treatment[1]	Sampled parameter	Treatment	Sampled parameter
P_1	C	faeces	C	faeces
P_2	C	ileal digesta		
P_3	C′	faeces	C′	faeces
P_4	C′	ileal digesta		
P_5	A	faeces and ileal digesta		
P_6	C′	ileal digesta		

[1] Treatment A and C were similar to those used in Exp. 6. Diet C′ was fed in equal amounts Hex. Eq. per kg BW as diet C.

Measurements and analyses:

a. Diet composition and daily feed intake.
b. Faecal characteristics
   - visual score,
   - pH.
c. Urine excretion in P_1, P_3 (fistulated animals)
   - weight,
   - reducing substances content.
d. Ileal digesta flow rate
   - weight of wet digesta,
   - osmolality.
e. Ileal and faecal samples analysed on
   - DM, ash, N, EE and carbohydrate composition.

Diet composition[1]*:*		Diet A (%)	Diet C (%)	Diet C′ (%)
skim milk powder		66.6	53.5	6.9
lactose hydr. skim milk powder[2]		–	–	46.9
lactose		13.9	41.8	–
dextrose		–	–	20.9
galactose		–	–	20.9
fats + lecithin		18.2	3.6	3.4
premix		1.2	1.0	1.0
Fe premix		0.1	0.08	0.08
Chemical composition:				
DM	%	97.4	97.4	95.5
CP (N × 6.38)	%	24.1	20.2	19.5
EE	%	18.2	3.8	3.8
NFE	%	49.2	68.6	67.6
lactose	%	45.7	66.8	3.4
ME	MJ/kg	18.4	15.4	15.2

[1] For detailed information see Exp. 6.
[2] Lactalac M®; C.C.F., Leeuwarden.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: Ileal digesta and faeces were collected over 5 × 12 and 5 × 24 h, respectively. Individual samples were analysed by method I and aliquot samples for each treatment according to OLLING (1972).

EXPERIMENT 15.

THE EFFECT OF LACTOSE INTAKE ON THE ABSORPTION AND RETENTION OF MACRO ELEMENTS.

Number of calves: 20; 10 fitted with re-entrant ileal cannulae.
Age at start: 5 weeks.
Experimental periods: 3 × 5 days; 2 days recovery between each period.
Experimental design:

Exp. period	Fistulated calves		Non-fistulated calves	
	Treatment[1]	Sampled parameter	Treatment	Sampled parameter
P_1	A	ileal digesta	A	faeces, urine
P_2	B	ileal digesta	B	faeces, urine
P_3	C	ileal digesta	C	faeces, urine

[1] For detailed information see Exp. 6.

Measurements and analyses:
a. Daily feed intake.
b. Faecal characteristics – visual score,
– pH,
– weight.
c. Urine excretion – weight,
– glucose and galactose content (method II).
d. Ileal flow rate – weight of wet digesta,
– glucose and galactose content (method II).
e. Na, K, Cl, Ca, P and Mg analyses in all samples.

Diet composition[1]*:*		Diet A (%)	Diet B (%)	Diet C (%)
skim milk powder		65.8	57.4	52.3
lactose		15.4	32.2	43.0
fats + lecithine		17.5	9.3	3.6
premix		1.2	1.1	1.0
Fe premix		0.1	0.09	0.08
Chemical composition:				
DM	%	97.5	97.5	97.6
CP (N × 6.38)	%	24.3	21.7	19.8
EE	%	17.7	9.6	3.9
NFE	%	49.6	61.2	69.1
ME	MJ/kg	18.3	16.6	15.4
Na	%	0.33	0.30	0.28
K	%	1.22	1.03	1.00
Cl	%	0.76	0.73	0.62
Ca	%	0.97	0.86	0.77
P	%	0.76	0.67	0.66
Mg	%	0.14	0.12	0.12

[1] For detailed information see Exp. 6. Water used for the dilution of the substitutes contained 4.7, 6, 8, 23, 7 and 0 ppm, respectively, of the tested minerals.

Feeding frequency: Twice daily; 8^{00} and 20^{00} h.

Miscellaneous: Ileal digesta, faeces and urine were collected during 5 × 24 h in each period, except in P_3 when the animals suffered scours in such an extent that diet C was withdrawn after 3 days on treatment.

CURRICULUM VITAE

De auteur van dit proefschrift werd in 1936 geboren te Hallum. Na het behalen van het einddiploma HBS-B, ving hij in 1954 aan met de studie aan de Landbouwhogeschool te Wageningen. In 1961 werd deze studie afgesloten met de ingenieursvakken: Veevoeding, Veeteelt, Fysiologie der Dieren en Pluimveeteelt. Vanaf 1963 was hij werkzaam bij N.V. Philips Duphar, aanvankelijk als technisch-commeriëel medewerker voor de Benelux en vanaf 1966, als hoofd van de technische-commeriële afdeling bij de Hoofd Industrie Groep, biochemische afdeling. Eind 1969 heeft de auteur een functie aanvaard bij de vakgroep Veevoeding van de Landbouwhogeschool. In deze functie is hij, na enige jaren van voorbereiding, gestart met onderzoek gericht op de voeding van herkauwers en kalveren. In dit kader is het beschreven onderzoek verricht.