Towards a physiological feeding strategy for protein in broilers

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STELLINGEN

1. Bij het snel groeiende vleeskuiken wordt de behoefte aan aminozuren in het voer veel meer bepaald door het aminozuurpatroon van de aanzet dan door de behoefte voor onderhoudsdoeleinden.

Dit proefschrift.

2. De $^{14}CO_2$ ademtest voor aminozuren geeft meer informatie over de korte (orde van uren) dan de lange (orde van dagen) termijn benutting van aminozuren in het metabolisme.

Dit proefschrift.

3. De verteerbaarheid van nutriënten in grondstoffen voor veevoeders behoort zoveel mogelijk gemeten te worden bij dieren die vergelijkbaar zijn met de dieren die de uiteindelijke voeders moeten nuttigen.

Dit proefschrift.

4. Een maximaal stiktofrendement ontstaat niet bij een maximale groeisnelheid.

Dit proefschrift.

5. De vorming van urinezuur noodzakelijk bij een overschot aan stikstof in het dieet van pluimvee kan leiden tot een verhoogde behoefte aan glycine of threonine

Dit proefschrift.

6. Indien de inname van eiwit van de volwassen Nederlandse humane populatie gelijk zou worden gehouden aan de aanbevelingen zou een ongeveer gelijke reductie in stikstofuitstoot bereikt worden als bij totale afschaffing van de vleeskuikenhouderij.

Centraal Bureau Statistiek, 1994, De landbouwtelling 1994. Misset, Doetinchem.
Sipman, E.A.E, Giesen, G.W.J., Berentsen, P.B.M. & Antuma, S.J.F., 1994. Naar een duurzame landbouw in Noord-Brabant: kosten en baten voor boer en milieu. Deelrapport pluimveehouderij. Vakgroep Agrarische Bedrijfseconomie, Landbouwuniversiteit Wageningen, Wageningen.
Voorlichtingsbureau voor de Voeding, 1993, Zo eet Nederland, 1992. Resultaten van de Voedselconsumptiepeiling 1992. Voorlichtingsbureau voor de Voeding, Den Haag.

7. De veronderstelling van Cumming dat *choice feeding* beter is dan het voeren van volledige voeders kan grote gevolgen hebben voor de toekomst van het pelleteren van voeders.

Cumming, R.B., 1994, The biological control of coccidiosis by choice feeding. Proc. 19th World's Poultry Congress, Volume 2: 425-428.

8. Gezien de voordracht van Quintanilha zijn hardlopers doodlopers want: Oxidative stress, such as invoked by exercise, might increase ageing.

Vrij naar: Quintanilha, A., 1995, Oxidative pathways in biology and medicine. Proc. VII Symposium on Protein Metabolism and Nutrition, Estaçâo Zootécnica Nacional, Portugal.

9. Gezien de moeizame financiering van fundamenteel onderzoek wordt in Nederland onvoldoende onderkend dat praktisch gericht onderzoek door fundamenteel onderzoek moet worden ondersteund.

10. Alleen in de wetenschappelijke wereld gaat een promotie vaak gepaard met werkeloos worden.

11. De stelling dat mooi ondergeschikt mag zijn aan functioneel geldt in sterke mate voor dijken.

12. De benaming 'vlees'kuiken of 'braad'kuiken gaat voorbij aan het feit dat het dier eerst geslacht moet worden voordat het vlees gebraden kan worden.

13. Een beetje AIO is een beetje eigenwijs.

Rob A.H.M. ten Doeschate
Towards a physiological feeding strategy for protein in broilers
Wageningen, 20 juni 1995

aan mijn moeder
en
ter nagedachtenis
aan mijn vader

CONTENTS

GENERAL INTRODUCTION

Importance of nitrogen efficiency in intensive animal production

Historically, animal production for supplying high quality food for human consumption was enabled because animals can utilise low value food and change that into high value protein products (milk, eggs, meat). When animal production appeared to be more profitable than selling plant products directly more area was used to produce fodder crops (Bieleman, 1994). With improving knowledge of animal nutrition people learned to provide animals with better diets based on mixtures of foodstuffs such as cereals, legumes and by-products. This improved animal production. Farmers specialized either in animal production or in plant production resulting in an intensive animal production independent of agricultural area. In present intensive animal production still a substantial part of the foodstuffs used are residues from human food production. Some examples are soybean meal (from soya-oil production), beet pulp and molasses (from sugar production), wheat by-products (from wheat flour production) and corn gluten (from corn starch production). For some other foodstuffs animal use however is in direct competition with human use. Some examples are: wheat, corn and tapioca. For this reason in periods of local food shortage (World War I and II) animal production was reduced (Bieleman, 1994).

After the second world war (1940-45) animal production increased in order to supply the increasing demand for animal products. To feed these animals the import of foodstuffs was necessary. Economically this import is more than compensated for by the export of animal products. However, in The Netherlands

the import of minerals in foodstuffs is not compensated for by the export of minerals in animal products or otherwise. On the long run a build-up of minerals in the environment will result and may eventually reach pollution levels. Mineral pollution could be reduced either by a reduction of pollution per amount of animal product produced or by a reduction in animal production. In both situations the balance between import and export of minerals should become more even. Because of the economical importance of animal production there was political consensus that it should be aimed for to reduce environmental pollution from animal production as a whole while keeping total production at the running level.

Although several minerals contribute to the pollution problem, the present discussion is restricted to nitrogen. Nitrogen is excreted by animals as apparent undigested nitrogen in the faeces and as urea (mammals) or uric acid (birds) in the urine. Subsequently, depending on the conditions in the manure and after application to the fields, the excreted nitrogenous products are degraded to nitrate and/or ammonia. This pollutes the environment in two ways: Firstly, nitrate leaches from the soil to the water in case of a too high amount of nitrogen for plant growth applied to the soil. Secondly, ammonia emission into the air results in acid deposition (Asman, 1987). Both these problems should be tackled. In the Netherlands, it was chosen to study two complementary ways to solve the pollution problems. The first way was to improve management of manure and the second was to improve nutrition.

The research concerning better management of manure resulted, amongst other technical measures, in improved housing systems with less ammonia emission for broilers (Leenstra & Ehlhardt, 1994). Further on application of manure to the land is improved so less nitrogen will be transformed to ammonia and more will be available for the plants. However, this more or less results in a transformation of air pollution (ammonia) to water pollution (nitrate). So, although technological research has resulted in more efficient handling of manure, the problem of a too high nitrogen application remains. Of course, more efficient handling of manure (reduced losses in barn, during storage and during application to the field), will lead to more nitrogen reaching the plants. Nitrogen from manure can thus be more efficiently used for plant production. This should result in less artificial fertilizer applied to the fields and thus less nitrate leaching. In this way better management of manure leads to less ammonia emission and less nitrate leaching.

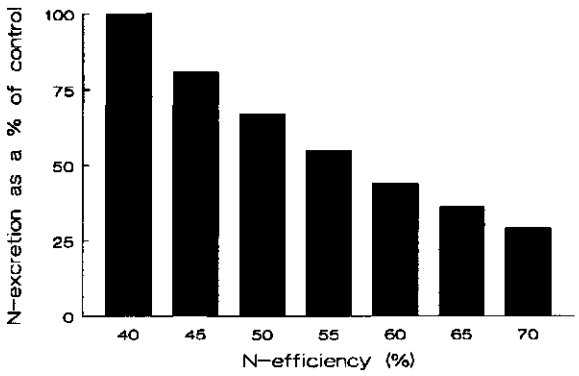

Figure 1. Effect of N-efficiency on excretion of nitrogen at a fixed production level. N-excretion is given relative to the N-excretion at 40 % N-efficiency.

The nutritional approach to reduce the excess nitrogen excretion intends to improve the efficiency of nitrogen utilisation in animal production. The objective is to attain a similar production level with a lower nitrogen input an thus a lower N-excretion. Nett, a lower nitrogen pollution level will be reached. Improvement of nitrogen efficiency in a nutritional way results in a more than equal reduction in N-excretion. If for instance N-efficiency is 40 % then a 10 % increase up to 44 % will result, at a fixed production level, in a reduction of excreted nitrogen of 15 % (60 gram excreted per 40 gram produced versus 51 gram excreted per 40 gram produced). This is illustrated in figure 1.

Improvement of the efficiency of the transition of dietary (mostly vegetable) proteins to animal protein has been an important subject of research previously in the sixties and seventies when protein was, relative to energy, an expensive nutrient. For this reason alternative N-sources were used (Featherston et al., 1962) which resulted in an improved utilisation of plant protein but not in improved nitrogen efficiency. For the present problems, ie excess nitrogen excretion, a different approach is needed. Nitrogen efficiency should be increased as high as feasible. Theoretical maximal efficiency is not well described because underlying processes are not well understood. Tamminga & Verstegen (1991) based on several sources (ARC, 1981; Fisher, 1983; Oldham, 1987) state that 15-20 % of total absorbed N-input is unavoidably lost in protein synthesis in body, eggs or milk.

Biochemical basis for N-efficiency

Biochemically it is hard to understand why nett protein synthesis as such should have a certain level of inefficiency. If the system for protein-synthesis is available (t-RNA, m-RNA, ribosomes) then synthesis runs without amino acid losses. When an amino acid is necessary for protein synthesis one molecule is combined with one

t-RNA-molecule and transferred to the ribosome where it is combined with other amino acids to constitute the protein. In this process losses of amino acids are not likely. The inefficiency of the process of protein synthesis could then be ascribed to protein turnover. However, the process of protein turnover *per se* also has no intrinsic and fixed inefficiency. When the protein is broken down, the constituting amino acids are released into the free amino acid pool. From the free amino acid pool amino acids can again be used for protein synthesis. Thus, also in these processes no losses are likely to occur.
However, for some amino acids this argument does not hold. For instance, after incorporation in proteins histidine is partly transformed to methyl-histidine which can not be re-utilized for protein synthesis. For most amino acids however, utilization of amino acids for a certain process should not be described as a process with a certain level of efficiency. Each biochemical process either produces a desirable product and is thus 100 % efficient or it catabolizes amino acids and is thus 100 % inefficient for production. Arithmetic efficiency of utilisation of amino acids (or nitrogen) for nett protein synthesis (further referred to as production) is the result of the combination of these totally efficient or inefficient processes. These processes together constitute a growing animal.

Research should be able to pinpoint which of the total inefficient processes are necessary and thus constitute a non-productive requirement and which of those processes function to catabolize amino acid excesses. Improvement of nitrogen efficiency should be based on an understanding of the processes which result in a certain level of nitrogen efficiency. For this reason the Dutch Fund for Manure and Ammonia research (FOMA) financed the development of a project (physiological feeding strategy) to decrease nitrogen excretion based on a biochemical approach of the N-efficiency in animal production. It was chosen to study broiler chicks for several reasons. Experiments with broiler chicks don't take much time because of their high inherent growth rate. Broiler chicks are convenient experimental animals in that it is possible to use large numbers without financial or other problems. The aim of the project thus was to develop a physiological protein feeding strategy for protein to reduce nitrogen excretion in broiler production, which could also serve as a model for other types of animal production.

Ways to increase nitrogen efficiency by improved nutrition

At the level of nutrition several ways have been proposed to improve nitrogen efficiency. A simplified scheme of the transition of nitrogenous dietary compounds into animal product is shown in figure 2. This figure shows three points were efficiency of the transition could, theoretically, be improved.

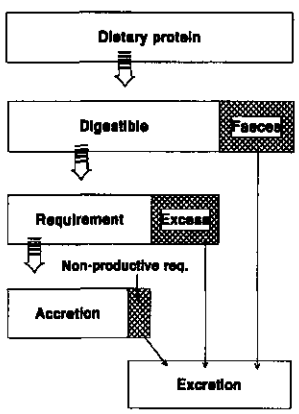

Figure 2. Possible losses in nitrogen transfer from diet to animal product.

Firstly, digestibility could be improved. There are several ways conceivable to compose a more digestible diet. These include choice of better digestible ingredients, addition of enzymes and diminishing of action of anti-nutritional factors (ANF's). The choice of better digestible ingredients within given foodstuffs depends on the availability of better digestible varieties, so breeding better digestible plant cultivars could be taken under this denominator. ANF content of foodstuffs could be lowered either by growing low ANF varieties or by technological treatment of foodstuffs.

Secondly, in most practical diets there will be an obvious mismatch between requirement and dietary supply for most amino acids. The clear reason for this is that the transfer of nitrogen from plant to animal is not the purpose for which the protein is synthesized. Plant proteins are synthesized as structural or reserve proteins for the plant, and not as foodstuffs for animals. In those rare cases where a protein is synthesized with the 'sole' purpose to transfer nitrogen, efficiency is very high. For instance the transfer of egg protein to chick protein was calculated to have an efficiency of 87 % (Schreurs et al., 1994). A similar example is the efficiency of body accretion in young mammals fed milk protein (Scheele, 1972). A 100 % efficiency is not attained nor expected. One reason for this is that other demands, such as physicochemical properties of the protein, limit the composition of the protein. For instance, protein solubility, structure and antibiotic properties

might impose a certain composition of the total egg protein. If the dietary amino acid composition is adjusted in a way to supply exactly the required amount of the separate amino acids then there will be no excess amino acid supply to contribute to the nitrogen excretion. Thus nitrogen efficiency will improve. Selection of dietary ingredients in combination with more extensive use of synthetic amino acids in diet optimalisation could result in better adjusted diets. Exact knowledge of amino acid requirements is a prerequisite for this kind of diet optimalisation.

Thirdly, there exists a gap between the amino acids deposited in the animal product and the amino acids required during production. The losses at this level might be called maintenance, or better, non-productive requirements (Schreurs et al., 1994). It is questionable to which extent these non-productive requirements for amino acids (which cause an apparent inefficiency of amino acid deposition) might be unavoidable. It should be considered whether the feeding strategy used will affect these non-productive requirements and thus N-efficiency in a positive or negative way.

Summarising, there are three points in the transition of dietary nitrogen to animal nitrogen where research might be able to improve the efficiency of transition. In the present thesis especially the second and third point will be matter of interest. The subject of this thesis can thus be limited to improvement of the productive utilisation of amino acids available from the diet in broiler chicks.

Functions of amino acids in metabolism

It is necessary to discuss the definitions of essential and non-essential amino acids in a study concerning amino acid utilisation. Therefore, in advance of a description of the function of the amino acids, the definition of essentiality is dealt with.
Amino acids are the constituents of proteins and are thus necessary for protein synthesis. The fixation of amino acids in protein is a all or none process. All amino acids constituting a protein to be synthesized must be available at the protein synthesis site simultaneously. If one amino acid is missing the synthesis of the whole protein is blocked resulting in excess of all other amino acids. Consequently the other amino acids will be redirected to other pathways, generally resulting in increased amino acid degradation. Thus for protein synthesis, at the synthesis site, all amino acids are equally essential. However, the common definition of essentiality is concerned with the question whether or not it is essential to supply

Table 1. Precursor or other functions of amino acids outside protein synthesis or direct energy production.

Alanine	(Ala)	N-carrier
Arginine	(Arg)	-Diamines/polyamines (putrescine, spermidine, spermine), ornithine, NO, creatine -Urea cycle
Aspartic acid	(Asp)	-Purine/pyrimidine synthesis -β-alanine -> panthotenic acid (CoA) (Microbial)
Asparagine	(Asn)	-See Asp
Cysteine	(Cys)	-Glutathione, taurine
Glutamic acid	(Glu)	-Central in N-metabolism -Glutathione, GABA, Gln, γ-carboxyglutamate -Arg, Pro, ornithine, citrulline (Not in birds)
Glutamine	(Gln)	-Amide of nicotinamide -Purine/pyrimidine synthesis -Regulation acid-base balance/urine pH
Glycine	(Gly)	-Glutathione, porphyrin, creatine -Purinesynthesis -Detoxification
Histidine	(His)	-Histamine, carnosine, homocarnosine, anserine -Imidazole propionic acid in skin: UV-protection
Isoleucine	(Ile)	
Leucine	(Leu)	
Lysine	(Lys)	-Carnitine, cadaverine
Methionine	(Met)	-Methyldonor -Cys
Phenylalanine	(Phe)	-Tyr
Proline	(Pro)	
Serine	(Ser)	-Gly, phospholipids, sphingolipids, ethanolamine, choline -Supplier one-carbon units
Threonine	(Thr)	-Gly, Ser
Tryptophan	(Trp)	-Serotonin -Nicotinamide -> NAD, NADP
Tyrosine	(Tyr)	-Thyroxine, melanin -Catecholamines, dopamine, (nor)adrenaline
Valine	(Val)	

Based on: Bender (1985) and Stryer (1988).

the amino acid with the diet. Whether an amino acid is called essential or not thus depends on the definition used. The most exact definition of an essential amino acid is an amino acid which can not be synthesized in the body and must thus be supplied by the diet. An amino acid which can be synthesized in the body out of another, essential, amino acid is called semi-essential. It is only essential to supply this amino acid with the diet if the supply of the substrate of which it can be synthesized is not sufficient. Amino acids which in principle can be synthesized in the body out of non-amino acid substrates but for which under certain conditions synthesis capacity is insufficient are called conditionally essential. In a way each amino acid is conditionally essential since without a suitable N-source and energy source it is impossible to synthesize a non-essential amino acid. In animal nutrition the definition of essentiality is a more practical one: An amino acid is essential if under normal conditions dietary supply of the amino acid is necessary for normal growth. In this context the limiting amino acid is the amino acid of which extra supply enables extra growth. Semi-essential is defined as being dependent on the availability of a certain substrate, such as tyrosine and cysteine (synthesized out of phenylalanine and methionine respectively). In nutrition, the distinction made between essential and non-essential amino acids is not always that clear. It was shown by Graber and Baker (1973) that glycine and proline were conditionally essential in young broilers for maximal growth due to insufficient synthetic capacity. *It should also be recognized that non-essential does not mean not* important. Probably non-essential amino acids can and will be synthesized in the body because they are so essential in the functioning of the body (Bergner, personal communication). When one considers the large role of the non-essential amino acids as intermediates in metabolism this statement sounds reasonable. For the whole body amino acids can be non-essential, essential or conditionally essential but at tissue or cell level the amino acids necessary for a certain function are essential. On an organism level, non-essential amino acids are only non essential if synthesis rate is sufficient to supply the required amount for all functions.

Besides their function in protein synthesis, most amino acids have additional metabolic functions. Most amino acids can be used to supply energy although differences in route of degradation will cause some amino acids to be more suitable for this purpose than other amino acids. A number of metabolic functions of amino acids have been elucidated quite well in a qualitative sense. However, it can not be stated that our knowledge is complete. Table 1 gives some examples of metabolic functions for amino acids. It can be seen that several so-called non-

essential amino acids play an extensive role in intermediate metabolism (Ala, Glx, Asx). Glycine is a very special case because it serves as a precursor for uric acid which in birds is the end-product of nitrogen metabolism. Each molecule of uric acid (four nitrogen atoms) contains one molecule of glycine (Stryer, 1988). Thus, in uricotelic species such as birds on a molar basis 25 % of the amount of nitrogen to be excreted has to be available as glycine. Glycine can be synthesized in birds through several pathways (Bender, 1985) but rate of synthesis might be lower than required resulting in a net non-productive requirement for glycine or one of the precursors. This point will be further attended later.

The use of certain amino acids for functions other than protein accretion normally does not involve the amino acid as a whole. In these cases it would be preferable to speak about partial utilisation of amino acids. For instance, after de-amination, only the carbon skeleton could be used. In effect, this implies that a certain part of the molecule (the amino group) is still available for other purposes or, alternatively, that a suitable source of the carbon skeleton can be used to have an amino acid sparing effect. For instance, when methionine is used as a methyldonor, methionine hydroxy analogue (MHA) will do as well. This is supported by numerous experimentations comparing the efficacy of methionine or MHA supplementation in poultry (Saunderson, 1985). An other example is reduction in the non-productive requirement of tryptophan by niacin supplementation. The observation that only the C-skeleton is required is of interest for limitation of nitrogen supply because evidently when only the carbon skeleton is required the supplementation of the intact amino acid will lead to unnecessary nitrogen load. Whether this will exert a quantitative important influence on total nitrogen efficiency depends on the relative importance of the non-productive requirement.

An attempt to study the importance of individual amino acids for maintenance of the body is the study of Kino & Okumura (1986) who fed diets devoid of one single amino acid. With this technique it was shown that diets void of methionine caused more weight loss than diets void of lysine. Based on these data it was concluded that the maintenance requirement of methionine would be higher than that of lysine. The value of this type of experiments for normal, fast growing broilers is disputable. However, it gives an indication that non-productive requirements differ between amino acids.

In general, use of amino acids for other purposes than protein synthesis is important for the functioning of the body but considering requirement the

quantitative importance depends on the relative importance of nett protein synthesis and of other metabolic processes.

Methods of determining amino acid requirements

The definition of what are amino acid requirements is a tough one. Commonly one would say: 'The requirement is that amount of amino acid a bird needs, for instance on a daily basis, to attain a given level of production'. However, this definition is rather inexact. What does a bird need, especially for what purpose ? This can be taken back to the method to determine requirements, to which this paragraph is dedicated. A more general definition of requirement values is: An amino acid requirement is a figure stating which amount of an amino acid, relative to other amino acids or absolute, a bird should receive based on a single or a combination of response parameters. This figure is used for calculation of dietary composition on a basis of a given dietary intake.

There are several techniques used to determine amino acid requirement. Two principally different approaches can be distinguished. The first, classical, approach is to start with a basal diet lacking one or more amino acids to which the limiting amino acid is stepwise supplemented up to a maximal response level. The second approach is a factorial approach in which requirement is calculated based on utilisation of amino acids for productive and non-productive purposes.

Classical requirement studies

The main body of classical requirement studies involved dose-response experiments with diets similar to diets used in practice. The general principle of these experiments is that the amino acid under test is supplemented to a basal diet with all the other amino acids and energy at levels sufficient to ensure that they can not be limiting in the study. The point where performance is no longer improved by extra supplementation is taken as the requirement of the tested amino acid for the response parameter measured. The statistical model to determine the requirement point is either a broken line (or bent-stick) model or an exponential curve giving the relation between amino acid intake and performance. Theoretically one would expect a broken-line model while in practice often a more smooth curve is observed. This apparent discrepancy is explained by Fisher et al.(1973) in the

description of the so-called Reading model for laying hens. The integration of several individual broken-lines within a population slightly differing in response will lead to a smooth curve. This implies that with each determination of requirements slightly different results may be expected because the transition point is not a sharp point but more an area. Whether a requirement value should be taken very strictly or not depends on the change in response when values just above or under the indicated requirement are fed. Or in other words, how steep is the curve of response against dietary intake level and how does it behave after the transition point. Because of this, requirement values should be presented with some kind of sensitivity parameter. Subsequently, requirements interact with other factors like health, environmental factors (temperature, light), management and dietary composition.

Moreover, requirement values found might depend on the response parameters used. Some examples of often used response parameters are: growth rate, feed conversion ratio, breast meat production, N-efficiency, economic return etc. For methionine, requirement for maximal growth performance is lower than for minimal feed conversion ratio (Schutte & Pack, 1995). For lysine, requirement to attain maximal growth rate is lower than for maximal breast meat percentage (Holsheimer & Ruesink, 1993).

A particularly interesting response parameter is the $^{14}CO_2$ recovery after injection of [^{14}C]amino acids. Dependant on the combination of amino acid varied in the diet and the [^{14}C]amino acid injected this method is referred to as the indicator or auto-indicator method. In case of the auto-indicator method the labelled amino acid is also the limiting amino acid varied in the diet. $^{14}CO_2$ recovery will start to rise at the dietary level where the amino acid supply is no longer limiting. When the [^{14}C]amino acid is different from the limiting amino acid varied in the diet $^{14}CO_2$ recovery will decrease until the level where the supplemented amino acid is no longer limiting. The question is whether this response parameter primarily responds to short term changes in the metabolic amino acid pool or as often assumed to long term nutritional protein status. With the last response in mind, this technique has been used extensively in baby pigs by Bayley and co-workers (Ball & Bayley, 1984,1985; Kim & Bayley, 1983; Kim et al., 1983a,b).

Henry et al.(1988) discussed that requirements determined with $^{14}CO_2$ recovery as response parameter in general were somewhat lower than requirements based on more classic response parameters. As shown by Heger and Frydrich (1989),

requirement for maximal nitrogen efficiency may be lower than requirement for maximal production. Studies thus indicate that the requirement based on $^{14}CO_2$ recovery studies reflects the requirement for maximal efficiency rather than the requirement for maximal production. This is not surprising since the point which is taken as requirement is the point where oxidation of indicator amino acids reaches the lowest level while oxidation of the limiting amino acids starts to increase. So, over all, oxidation of amino acids will be minimal and thus oxidative losses will be minimal. This might result in maximal efficiency. For chicks some measurements are described by Bergner and co-workers (Bergner & Mnilk, 1991,1993; Bergner et al., 1987; Mnilk et al., 1993; Nhan et al., 1987; Wilke et al., 1987). In a following paragraph the phenomenon of metabolic amino acid utilisation, for the study of which [^{14}C]amino acids are well suited, will be discussed in more detail.

Factorial approach to determination of requirements

In the factorial approach, requirements are calculated based on the amino acid pattern of the product produced combined with non-productive requirements. This approach has been used by Hurwitz et al.(1978,1980) for broilers, Hurwitz et al.(1983) for turkeys, Bowmaker & Gous (1989) and Martin et al.(1994) for pullets. The amino acids required to form the product, ie the composition of body accretion in case of broilers, are quite easy to determine. However, determination of the non-productive requirements is less easy. In an ideal situation one could predict these based on the metabolic functions of the amino acids. So, for instance, non-productive requirement would probably be high for methionine (functions as methyldonor) while for lysine it would be low. However, as mentioned before, this methionine requirement is not for the amino acid as such but for the carbon skeleton of the amino acid. Based on lack of knowledge concerning the non-productive requirements authors tend to use the amino acid pattern of body protein (Martin et al., 1994). Further on, in the factorial approach mostly a certain level of efficiency of protein synthesis from amino acids is assumed (cf Hurwitz et al., 1978, Martin et al., 1994). As discussed previously, a fixed level of efficiency for all amino acids for protein synthesis has no biochemical basis. For most amino acids, non-productive requirements of amino acids should be based on other processes than protein synthesis and -breakdown. The question remains how to define (qualitative as well as quantitative) the non-productive requirements. Non-productive requirements can qualitatively be defined as catabolic processes running along with body functioning. To enable adaptive processes, a certain level of

metabolic degradation of essential amino acids is supposed to be unavoidable. In broiler chicks, however, the effect of non-productive requirements on the required amino acid pattern might well be negligible since, because of the high growth rate, most of dietary amino acid intake (60-70 % of apparent digested amino acids) is used for body accretion. So, for broiler chicks, the factorial approach might be simplified such that the dietary amino acid pattern should be based on the amino acid pattern of body accretion.

Concluding, with a bird's eye view one could distinguish two fundamental different approaches to amino acid requirement studies. The first approach is to determine requirement values for each amino acid separately by some sort of dose-response tests. The second approach is to compose a required dietary amino acid pattern based on a more or less factorial approach. The merits of both methods are point of discussion.

Estimates of amino acid requirements

Baker and co-workers did several classical requirement studies to determine requirements for a range of amino acids. This series of experiments culminated in an 'ideal' amino acid pattern for broilers (Baker & Han, 1994). This pattern is given relative to lysine and is based on the consideration that lysine is mainly used for protein synthesis and has a limited array of metabolic functions. Moreover, there is a substantial amount of knowledge concerning the requirement for lysine under a variety of circumstances. Austic (1994) in a recent review presented broiler amino acid requirements for some essential amino acids. The results of both Austic (1994) and Baker & Han (1994) are compared with latest NRC values (1994) in table 2.

Although all three sets of requirement values were based on different data the resulting required amino acid pattern is remarkably similar. This is especially true if it is realized that for composition of diets the contents of available amino acids in the foodstuffs should be known. Amino acid analysis has a quite high intrinsic variability while in practice most foodstuffs are not analyzed for amino acid contents but table values are used for diet optimalisation. This implies that it will be hardly feasible to compose diets which exactly supply the amino acids required. Considering this there seems to be a close agreement between the above mentioned authors concerning the required amino acid pattern. For all three sources the response parameter was similar: maximal growth rate combined with minimal

feed conversion ratio. For maximal nitrogen efficiency requirements might differ substantially.

Table 2. Recommended dietary amino acid pattern according to different sources. All amino acids are given relative to lysine = 100

	Austic (1994) [1]	Baker & Han (1994) [2]		NRC (1994) [3]	
		0-21 d	22-42 d	0-21 d	22-42 d
Lys	100	100	100	100	100
Arg	96	105	105	114	110
His	24	37	37	32	32
Ile	65	67	67	73	73
Leu	92	111	111	109	109
Met	38	36	37	45	38
Met + Cys	72	72	75	82	72
Phe + Tyr		105	105	122	122
Thr	62	67	70	73	74
Trp	18	16	17	18	18
Val	69	77	77	82	82

[1] and [3] : Based on level of amino acids in the diet, not corrected for digestibility.
[2] : Based on true faecal digestible amino acids.

Physiological feeding strategy

In practice, the feeding of broilers is nowadays directed more to maximal production than to maximal nitrogen efficiency. The feeding strategy used normally consists of a three phase feeding system. The first or starter phase, is from 0-14 or 21 days of age. The second or grower phase starts at the end of the first phase and lasts until about a week before slaughter. The last or finisher phase is used to feed diets without additives in order to supply broilers free from residues to the slaughter house. The length of this period depends on the withdrawal period

required for the feed additives. Feed composition is altered in each phase to accommodate changes in required protein/energy ratio. A further adjustment of the feeding strategy to the physiological and metabolical conditions of the birds might improve nitrogen efficiency. In short, this is our opinion concerning what a physiological feeding strategy is about.

Due to the high growth rate of broilers amounts of dietary amino acids required for non-productive purposes are much lower than amounts required for production (Fisher, 1983). The main part of dietary amino acid intake is thus required for nett protein synthesis. Thus, when the amino acid composition of body accretion changes then one would expect changes in the required dietary amino acid pattern. It is thus necessary to determine the amino acid composition of body accretion over small time-periods. Ideally the amino acid pattern of body accretion should be known continuously but experimental limits constrain this type of determinations to periods of a week. Amino acids used for non-productive purposes such as inevitable oxidative losses or losses in digestion, are the second large part of the required amino acids. A proper experimental way to determine these requirements has not been shown yet. A proportion of non-productive requirements is constituted by the amino acids lost in the process of synthesis, degradation and re-absorption of endogenous material in the intestinal tract. It is possible to correct for these requirements by calculating amino acids available from the diet on an apparent ileal digestible base. In this way, the losses of endogenous amino acids are considered to be related to the amount and specific properties of the dietary components eaten. Main losses take place in the metabolic utilisation of the amino acids. *In vivo* metabolic studies with labelled amino acids can be used to study these processes. Results of these tracer studies provide optimal conditions to improve the physiological feeding strategy. Further physiological or environmental conditions might also contribute to a physiological feeding strategy. Firstly, the management of the birds (housing, light regimen, feeding regimen, climate) will affect the nitrogen efficiency and should thus be taken into consideration or at least kept constant. Secondly, the capacity of the birds (genotype, sex) will also affect the ability to reach a high nitrogen efficiency. These conditions might also interact with the diet and should thus be studied. The physiological feeding strategy is thus concerned with all aspects of nitrogen metabolism and the factors affecting nitrogen requirement and nitrogen efficiency.

Metabolic amino acid utilisation

In vivo metabolic studies with labelled amino acids can be used to study metabolic utilisation of amino acids in intact animals without disturbing normal physiological processes. [^{14}C]amino acids are used to study the fate of the carbon skeleton. [^{15}N]amino acids are used to study the fate of the amino group. Other labels applied in studies of amino acid utilisation are ^{3}H and ^{35}S. Most commonly used are ^{14}C and ^{15}N amino acids. Within this thesis only the use of [^{14}C]amino acids is discussed.

Tracer substances can be used for several purposes. Two distinct types of measurements are of special interest for amino acid metabolism. The first is the measurement of protein synthesis by determination of the incorporation rate of a [^{14}C]amino acid. In recent years for this type of measurements the flooding dose technique (Garlick et al., 1980) is used. With this technique it is possible to measure the amount of tracer used for protein synthesis during a short period of time and thus the momentary protein synthesis rate. The second type of measurements is the end product measurement in which the excretion of $^{14}CO_2$ from [^{14}C]amino acids is measured. In this type of measurements the $^{14}CO_2$ recovery is a measure for the oxidation of the amino acid relative to other processes that use the C-skeleton.

This latter type of measurements is especially suited for studies related to efficiency of amino acid utilisation. For this reason the remainder of this paragraph is dedicated to this type of measurements. In our department this technique has been used to study metabolic amino acid utilisation in rats under different physiological conditions (Schreurs et al., 1992; Weijs, 1993).
Starting in the seventies (Colvin et al., 1969, Brookes et al., 1972) $^{14}CO_2$ recovery has been suggested as a way to determine amino acid requirements. Several approaches have been used while interpretation of results was sometimes not unequivocal. The requirement curve describes the relation between dietary amino acid supply and $^{14}CO_2$ recovery from tracer amino acids.

Neale & Waterlow (1974) discussed that, although specific activity of precursor at oxidation site was not known, measurement of requirement was correct since the inflection point of the requirement curve was determined after measurement of total recovery. This is only correct if specific activity is not affected by dietary treatment. If not, the $^{14}CO_2$ recovery should be corrected for the specific activity of the precursor pool. However, as discussed by Weijs (1993), any specific activity

measurement is at least arbitrary. Moreover, steady state is a necessary condition for efficient use of specific activity measurements. Metabolic amino acid utilisation is in essence not a steady state phenomenon so interpretation of measurements should preferably be made without a correction for specific activity of the precursor pool. When a single shot tracer dose is given the resulting $^{14}CO_2$ recovery is merely a reflection of the partitioning of amino acids between possible pathways of the labelled pool. In this case correction for specific activity is not relevant. If a whole body model with one free amino acid pool (Fig. 3) is used then this recovery reflects the relative importance of amino acid oxidation versus other metabolic processes, mainly protein synthesis.

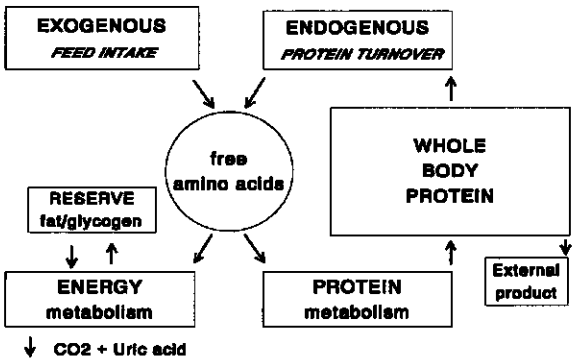

Figure 3. One-pool model of amino acid flux in the body (After Schreurs et al., 1992)

According to the one-pool model the labelled pool after injection is the total free amino acid pool, combining contributions of an endogenous part (from protein breakdown) and an exogenous part (dietary supply). With the single shot tracer method it is possible to study metabolic utilisation in a relative way as affected by physiological state. With animal production efficiency in mind the main interest is the metabolic utilisation immediately after a meal in fast growing animals. The maximal attainable efficiency of amino acid deposition is determined immediately after absorption of the amino acids. Amino acids oxidised at this moment can not contribute to protein synthesis at a later stage and are thus lost for protein synthesis.

Most metabolic studies have either been done in fasted animals after a single meal (Bayley, 1985) or in animals with relative low growth potential such as rats. Our interest is in the metabolic utilisation of amino acids in fast growing, well fed animals such as broiler chicks. Measurements of $^{14}CO_2$ after single shot [^{14}C]amino acid injections in birds have been made by Wang et al. (1973), Simonnet et al. (1989a,b) and Bergner et al. (1987). Based on these studies combined with the idea's of Bayley and co-workers we planned a series of experiments to determine whether it would be possible to use $^{14}CO_2$ breath test experiments to study

metabolic utilisation in broilers and to study effects of dietary amino acid concentrations. A similar setting was used by Buten (1989) to study the efficacy of different methionine supplements. Buten (1989) reached the conclusion that *complicated metabolic assays to evaluate methionine sources in broiler chicks on a cellular level demand as much time and costs as simple growth assays do.* This conclusion holds probably also for the use of $^{14}CO_2$ recovery studies to study amino acid requirements. However, $^{14}CO_2$ breath tests can give more insight in the reason for a certain performance and may thus, in the long run, result in a better understanding of the underlying processes. Especially short term metabolic losses related to certain conditions (e.g. relative to a meal or after physiological disturbance) could be subject of study. Also short term changes in requirement such as requirements for special purposes (e.g. feather synthesis at a certain age) can be perfectly studied. $^{14}CO_2$ breath test should thus be looked upon as an additional technique to study metabolic amino acid utilisation supplying extra information compared to long term performance studies.
Summarising, metabolic amino acid utilisation can be studied by *in vivo* metabolic studies with labelled amino acids. In fast growing broiler chicks the $^{14}CO_2$ breath test gives information concerning the utilisation of the amino acid under test for oxidation relative to other processes. The $^{14}CO_2$ breath test is suited to determine changes in short term utilisation of the carbon skeleton of amino acids.

Towards a new hypothesis to increase nitrogen efficiency

It is supposed that a certain level of amino acid oxidation is not related to the physiological situation. Oxidation of amino acids, as measured with the $^{14}CO_2$ breath test implies that the carbon skeleton of the amino acid is no longer available. However, the amino group is transferred to another carbon skeleton before excretion. In this way during breakdown of all amino acids, nett synthesis of non-essential amino acids occurs. The N released from catabolized amino acids is, in poultry, eventually converted to uric acid and excreted. The precursors of uric acid are glutamine, aspartic acid and glycine. This means that all nitrogen to be excreted passes the stage of non-essential amino acids before excretion. If these non-essential amino acids could be utilized for net protein deposition, N-efficiency would improve drastically. This hypothesis is based on the very efficient nitrogen transfer in the production of a chick out of an egg (Schreurs et al., 1994) and growth of rats on casein diets (Scheele, 1972). Scheele (1972) found that efficiency of deposition of total non-essential amino acids was higher than the

efficiency of each essential amino acid. These results were then interpreted as that non-essential amino acids were limiting in the casein diet. The present hypothesis suggests that nitrogen from degraded essential amino acids was used to synthesize non-essential amino acids thus resulting in a higher efficiency of deposition for non-essential than essential amino acids.

The hypothesis could be tested by lowering the content of non-essential amino-N in a diet where the pattern of the essential amino acids was adjusted to the pattern of body accretion. However, since only the normal products of transamination processes (glx + ala + asx) are available in excess, only these should be lowered. Proline and glycine should be fed according to the pattern of body accretion since these are not synthesised as products of normal amino acid degradation. The hypothesis that lowering non-essential N might result in higher N-efficiency through re-utilisation of excess amino-N deserves attention. Thus, the concept using the amino acid pattern of body accretion as basis for diet formulation, as put forward before, holds only for the essential amino acids and the non-essential amino acids proline and glycine. Theoretically this approach has the potential to improve nitrogen efficiency beyond the level which can be reached if all amino acids are supplied according to requirements for productive and non-productive performance. Carcass composition should be a point of attention, as it is shown that lower levels of NEAA result in increasing fatness (Moran et al., 1992).

Furthermore, the glycine required for uric acid formation is incorporated entirely. This implies that one quarter of total nitrogen excretion should be available as glycine. Glycine can be converted from serine which in turn can be converted from threonine (Bender, 1985). This results in a non-productive threonine requirement when there is a relative large excess of nitrogen and thus a large uric acid synthesis. So a second hypothesis is that Thr requirement is increased with increasing level of dietary nitrogen excess. A conceivable way to test this is to test whether dietary NEAA-level and Thr-level interact on production and N-efficiency performance. A practical implication of this hypothesis would be that threonine requirement could be lower in case of low dietary NEAA excess.

Outline of the thesis

This thesis is written as a report of a 4 year project concerning the improvement of N-efficiency in broiler production by development of a physiological feeding strategy. The chapters describing part of the project are more or less in chronological order. Within the limits of the project the chronological order also is a logical one. The first chapter describes the assessment of the amino acid pattern of body accretion as basis for the required available amino acid pattern. This pattern is studied in both sexes of chicks of three genotypes differing in growth potential and efficiency. A possible change of the pattern with age, combined with effects of strain and sex, was the main aim of this study. In chapter 2 two aspects of digestibility are discussed. First, it is studied whether digestibility is affected by genotype, age or sex of the birds used. Secondly, the method to determine amino acid digestibility is discussed. Correct assessment of digestibility (as an estimate for availability) of dietary amino acids is very important to be able to do research concerning utilisation of available amino acids. For this reason this chapter is dedicated to digestibility studies in broiler chicks. Based on this chapter, a standardized protocol was used to determine ileal amino acid digestibility coefficients of several foodstuffs (Scheele et al., 1992). These coefficients were used to calculate apparent ileal digestible amino acid content of the diets used in further studies. Chapter 3 contains the first report of $^{14}CO_2$ breath test measurements as performed within this project. It mainly discusses injection method and time of injection relative to a meal. In chapter 4 the $^{14}CO_2$ breath test measurement is used to determine dietary influence on amino acid utilisation. It is studied whether dietary amino acid level and the length of the pre-experimental conditioning period on the diet affects amino acid utilisation as measured with the $^{14}CO_2$ breath test.

Chapter 5 describes an experiment in which nitrogen efficiency is determined when diets with an amino acid pattern based on body accretion are fed to male or female birds of two genotypes. Furthermore, variation in dietary protein/energy ratio and in amino acid supplementation were studied.

In chapter 6 nitrogen efficiency of birds fed diets based on body accretion or other reference amino acid patterns is discussed together with an experiment in which both hypotheses described in the previous paragraph were tested. In chapter 7 the slaughter results of these experiments are reported. The thesis is then concluded with a final discussion chapter. In this final chapter, all experiments are discussed and the potential for improvement of N-efficiency is discussed in a wider context.

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Chapter 1

WHOLE BODY AMINO ACID COMPOSITION OF BROILER CHICKS AS DETERMINED BY AGE, GENOTYPE AND SEX.

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Summary

Whole body amino acid composition of broiler chicks was studied with the aim to provide a basis amino acid pattern for diet formulation. Three genotypes were studied; a commercial broiler hybrid (Com) and two experimental lines: one selected for high growth rate (GL) and one selected for efficient feed conversion (FC). Male and female Com chicks were compared. Change of whole body amino acid composition with age was studied in male Com chicks by weekly analysis.

Whole body amino acid composition at the consecutive sampling moments showed several small, but statistically significant ($P < 0.001$), differences. Differences in whole body amino acid composition, larger than 10 % of the mean were found for cystine (7 vs. 14, 21 vs 28 and 28 vs 35 days of age), glycine (21 vs 28, 35 vs 42 and 42 vs 49 days of age), alanine (7 vs 14 and 21 vs 28 days of age), histidine (21 vs 28 days of age) and tryptophan (35 vs 42 days of age). For some amino acids there was a gradual shift in the relative amino acid content with age in which during the first three weeks content was either lower (cys, asp, thr, ser, ile, leu & phe) or higher (gly, ala) than during week 4-7. The differences in the amino acid pattern of body accretion in 0-21 and 22-49 days of age leads to the proposal to compose different dietary formulations in these age-periods.

The three genotypes differed in relative amino acid composition. Analysis of growth pattern revealed no differences between Com and GL genotype, the FC genotype however had a clearly different growth pattern. Similar growth patterns did not result in similar whole body amino acid composition.

Compared to male Com birds, whole body of female Com birds contained relative less essential amino acids (cys, met, val, tyr, phe and his) and more non-essential (glu, asp) amino acids. The amino acid pattern of whole body accretion in male Com chicks can be used as a basis for diet formulation for broiler chicks of different genotypes and sexes.

Introduction

To obtain a high nitrogen efficiency in broiler production, it is important to adjust the dietary amino acid supply to the requirements. Whole body amino acid accretion is supposed to be a good basis for estimation of dietary amino acid requirements, when maintenance requirements are also accounted for (cf. Hurwitz et al., 1978). In broiler chicks, the amino acid requirement for net growth is large compared to requirements for maintenance (Baker & Han, 1994). We therefore assume that the amino acid pattern of body accretion largely determines the required dietary amino acid pattern. With age, the amino acid pattern of whole body accretion has to be studied because of differences in the allometric growth of body components. For instance, feather growth occurs in a distinct age period. For a correct description and modelling of whole body amino acid accretion of the broiler, we studied changes in body amino acid composition with age until slaughter (49 days of age). In 1988, Kreuzer et al. already reported the whole body amino acid pattern of broilers at 0, 14, 21 and 35 days of age. In the present study, modern broilers with a higher growth potential were studied. Our data are intended to be used as basis for the development of a 'physiological feeding strategy to improve nitrogen efficiency'. For a general application of such a 'physiological feeding strategy', it is important whether and to which extent differences in sex and/or genotype also affect whole body amino acid composition. The effect of sex was studied in male and female chicks of a commercial broiler line. Possible genetic differences were investigated for three genotypes with large differences in growth characteristics. The aim of this study was to determine a general dietary amino acid pattern for modern broilers based on data for amino acid composition of the whole body and that of body accretion.

Materials and methods

Birds and husbandry

Male and female chicks of three broiler genotypes were studied. The first genotype (Com) was a commercial broiler hybrid (Euribrid, Boxmeer), from a feather-sexable descent. Chicks from two experimental broiler sire lines were taken

from the 11th generation of the selection experiment described by Leenstra & Pit (1987). The GL line had been selected for high body weight at 42 days of age (GL line). The FC line had been selected for efficient feed conversion between 21 and 42 days of age (FC line).

All birds received the same diet consisting of corn, soybean meal, wheat, tapioca, soybeans, animal fat, soya oil, fish meal, feathermeal and sunflower meal. The composition and digestibility of this diet as affected by genotype, age and sex was fully described by ten Doeschate et al. (1993). The diet contained: dry matter 910 g/kg, GE 18.5 MJ/kg, mean AME 13.6 MJ/kg, CP (N*6.25) 217 g/kg, lys 11.1, met 6.3, met + cys 1.05, thr 0.86 and his 0.64 g/kg.

Day-old chicks were sexed, wing-banded and vaccinated against infectious bronchitis and Newcastle disease. At 17 days of age birds were vaccinated against bursal (Gumboro) disease. All chicks were housed in 72 litter floor pens (75 * 97 cm) divided over six environmentally controlled rooms. Environmental temperature decreased gradually from 32 °C for one-day old chicks to 18 °C at 6 weeks of age. After 3 days of continuous light, birds were kept under a lighting regime of 1L:2D in order to reduce the occurrence of leg disorders. Birds had continuous access to feed and water. Two replicate pens of 25 chicks in each room were used for each genotype-sex combination. The results of the two pens with the same treatment in one room were pooled to form one of six replicates. In the statistical analysis, room was used as the factor block.

Measurements

Weekly, starting at 6 d of age, three randomly chosen birds were taken from each pen for analysis. Total weight of the birds in each pen was determined before and after these birds were removed. Within a room, groups of birds were weighed each week at the same time. Feed consumption was determined each week. From the six birds out of the two replicate pens in one room, three were used for whole carcass analysis, while the other three were used for analysis of body components (to be presented in a subsequent paper). The birds to be used for carcass analysis, were housed in separate pens during the remainder of the day. These birds had feed and water available *ad libitum* until late in the evening. To empty the gastro-intestinal tract, feed was removed between 23.00 and 01.00. The following morning (07.30) these birds were taken to the slaughter facility, where they were euthanized with CO_2. All birds from one room were killed within a period of 45 minutes to avoid differences in fasting time (between 8 and 13 hours for the

different rooms) to be confounded with treatment effects. Immediately after killing, weight of the birds was recorded. Birds meant for total carcass analysis were stored at -18 °C until further sample preparation. For determination of the initial whole body amino acid composition from each genotype/sex combination 2 * 10 day-old chicks were euthanized with CO_2 and frozen immediately.

Sample preparation and analysis

Sample preparation was according to a modification of the method described by Scheele & Jansen (1971). Three whole carcasses were minced in a meat cutter while still frozen. A subsample (max 1000 g) was mixed with celite (to bind and disperse fat) and thereafter freeze-dried. After freeze-drying, all samples were ground to pass a 1.5 mm sieve.

For amino acid analysis, an acid and a separate oxidative hydrolysis were performed; trp was determined after hydrolysis under N_2. After passage through a LC-ion column (Beckman LC 5001) amino acids were derivatised with ninhydrin . Norleucine was used as internal standard. The estimated contents of thr, ser, ile and val were corrected for incomplete recovery after hydrolysis according to Slump (1969) and thus multiplied by the following factors: 1.05, 1.10, 1.08 and 1.07 respectively. Amino acid contents were expressed on a weight basis (g/100 g) relative to total amino acids recovered.

Statistics

Data were analyzed using Genstat as a statistical package (Genstat 5 committee, 1987, 1993). Methods of analysis are given below for different groups of data.

1) Growth and feed conversion data: Data of both replicates within a room were combined to constitute one experimental unit. Feed conversion ratio (FCR) was calculated per pen as mean feed intake per day per bird divided by mean daily growth per bird. Room was used as block factor. For each age, data were analyzed with ANOVA according to the following model:

$Y = \mu + \text{block} + \text{genotype} + \text{sex} + e$ {Model 1}

in which: Y = parameter under test, μ = mean, block = effect of factor block (room 1-6), genotype = effect of factor genotype (Com, GL or FC), sex = effect of factor sex (male or female).
For a more thorough analysis of growth pattern by age (wks) a model composed of both a linear increase in growth and an exponential increase was fitted. The function used was:

$$Y = a + b * x + c * r^x + e \qquad \text{[Function 1]}$$

in which: Y = growth; a = constant; b = slope for the linear component; c = weight factor for exponential part of the curve; r = basal value for exponential part; x = age (weeks); e = random error.

Groups of data, according to genotype/sex as factor values, were fitted against week as the explanatory variable. Differences in observed curves were determined through parallel curve analysis (Genstat 5 Committee, 1993).

2) Amino acid composition data: In male chicks of Com genotype, the effect of age (7-49 days) on amino acid pattern was tested for each amino acid according to the following model:

$$Y = \mu + \text{block} + \text{age} + e \qquad \text{\{Model 2\}}$$

If a F-test indicated an effect of age, then contrasts between ages were tested with a t-test based on regression of available data against age (Procedure RPAIR, Genstat procedure Library Manual Release 2 (1)).
The following analyses were performed using the procedures REML and PAIRTEST (Genstat 5 Committee, 1993) to determine the F-probability and t-tests respectively. For the determination of the effect of genotype and age on whole body amino acid composition, data of male birds of Com, FC and GL genotype at 14 and 49 days of age were analysed according to the following model:

$$Y = \mu + \text{block} + \text{genotype} + \text{age} + \text{age*genotype} + e \qquad \text{\{Model 3\}}$$

For the determination of the effect of sex, analysis of male and female birds of Com genotype at 14 and 49 days of age was made according to the model:

$$Y = \mu + \text{block} + \text{sex} + \text{age} + \text{age} * \text{sex} + e \qquad \text{\{Model 4\}}$$

If the F-test indicated a significant effect of a factor, then contrasts between groups were tested with a two-sided t-test.

Table 1. Growth rate (grams per bird per day) and feed conversion ratio (gram feed per gram gain) in seven consecutive weeks in both sexes of three genotypes (Com, GL and FC).

Age (d):		0-6	7-13	14-20	21-27	28-34	35-41	42-48
(Wk)		1	2	3	4	5	6	7
Growth rate								
Com	m	13.6	30.7	50.4	64.7	78.1	82.8	79.0
	f	13.6	29.4	44.7	54.2	62.7	64.1	61.4
GL	m	15.6	35.0	55.5	72.0	86.1	85.0	84.9
	f	15.5	32.5	50.6	62.8	71.9	72.5	66.9
FC	m	10.3	17.9	29.2	44.0	60.5	69.4	75.7
	f	10.4	18.1	29.3	42.8	54.9	60.9	57.8
significance levels								
Geno		***	***	***	***	***	***	***
Sex		NS	***	***	***	***	***	***
G * S		NS	**	***	***	***	***	NS
Feed conversion ratio.								
Com	m	1.13	1.29	1.37	1.51	1.64	1.83	2.09
	f	1.13	1.32	1.42	1.60	1.77	2.06	2.30
GL	m	1.11	1.30	1.36	1.49	1.64	1.97	2.11
	f	1.10	1.30	1.38	1.58	1.81	2.13	2.48
FC	m	1.15	1.37	1.41	1.42	1.44	1.59	1.70
	f	1.14	1.36	1.39	1.44	1.51	1.68	1.92
significance levels:								
Geno		***	***	*	***	***	***	***
Sex		NS	NS	*	***	***	***	***
G * S		NS	**	**	*	*	**	**

m = male; f = female; Com = commercial broiler hybrid; GL = line selected for high growth rate; FC = line selected for low feed conversion ratio; Geno = main effect of genotype; Sex = main effect of sex; G * S = interaction between genotype and sex
F-probability: * < 0.05; ** < 0.01; *** < 0.001

Results

Table 1 shows the mean body weight gain and feed conversion ratio (FCR) per week. The differences between genotype are significant for both parameters at every age. Sex has a significant effect on growth from the second week onwards, whereas for FCR sex had a significant effect from the third week onwards. Interactions between genotype and sex are caused by the relative small differences between male and female chicks in the FC line, compared to the other two genotypes.

Table 2. Coefficients of fitted curves for growth rate (gram) against age (wk). Function used: line plus exponential; fitted separately for all genotype/sex combinations. (mean; se)

$Y = a + b * x + c * r^x$ (x=age)

	Parameters							
Geno/sex	a		b		c		r	
Com/male	-5.17	1.10	20.45	1.57	-1.28	1.17	1.729	0.184
GL/male	4.2	10.4	28.58	4.23	-13.2	12.0	1.371	0.114
FC/male	-5.37	1.05	12.56	0.327	$-3*10^{-9}$	$1.1*10^{-7}$	21.3	97.6
Com/female	33.2	54.1	27.44	9.48	-38.4	56.0	1.23	0.128
GL/female	7.5	10.6	25.38	4.26	-13.1	12.2	1.369	0.116
FC/female	-2.91	1.16	11.35	0.416	$-1.8*10^{-4}$	$6.2*10^{-4}$	5.19	2.44

In table 2, a summary of the analysis of curves fitted to the growth data is given. For FC-birds the weight factor for the exponential part of the curve (c) was not significant different from zero. From the process of fitting a standard curve (linear plus exponential) to the growth data, it was concluded, that for FC-birds addition of the exponential part to the curve did not

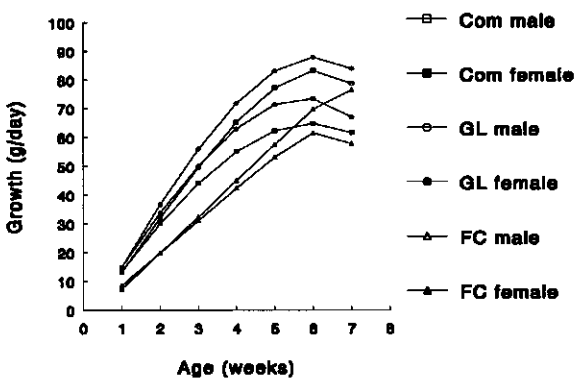

Figure 1. Fitted curves of growth rate against week of age according to a combination of a straight line plus exponential curve.

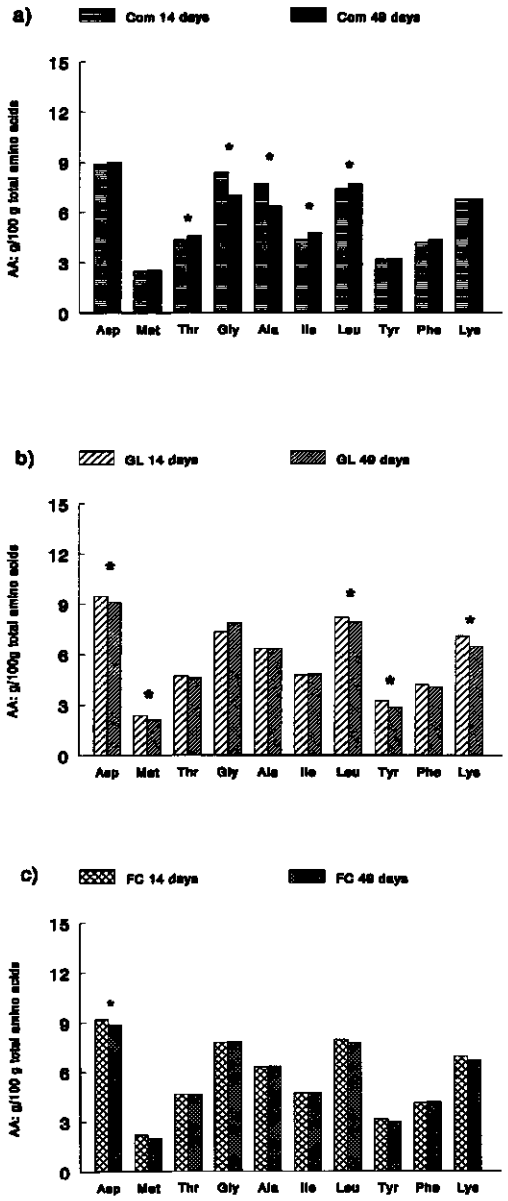

Figure 2. Amino acid pattern of birds at 14 and 49 days of age: a) Com, b) GL, and c) FC. Only amino acids where interaction between genotype and age was significant (F-test; $P<0.05$) are shown. * denotes significant contrasts ($P<0.05$).

improve the fit, whereas for the other genotypes -Com and GL- an exponential part did contribute to a better fit. Figure 1 shows that, until the last week, growth of FC birds increased nearly linear with time whereas other genotypes showed a decreasing slope of growth with time. Between Com and GL genotype or between sexes, there were no significant differences in curve parameters.

Table 3 shows amino acid pattern of the total carcass for male Com chicks at different ages. Differences were small but often statistically significant. Differences in whole body amino acid composition, larger than 10 % of the mean were found for cys (7 vs. 14, 21 vs 28 and 28 vs 35 days of age), gly (21 vs 28, 35 vs 42 and 42 vs 49 days of age), ala (7 vs 14 and 21 vs 28 days of age), his (21 vs 28 days of age) and trp (35 vs 42 days of age). Only met did not show any significant change with age. The relative content of some amino acids (cys, asp, thr, ser, ile, leu and phe) decreased from hatching up to 21 days of age, after which their relative content rose again. Other amino acids (especially gly and ala) showed a relative rise during the first 21 days, after which their relative content decreased again. These data indicate a general change in whole body amino acid composition around 21 days of age.

Table 4 shows whole body amino acid pattern of the different genotypes at 14 and 49 days of age. Significant interactions between genotype and age were present for 10 amino acids (asp, met, thr, gly, ala, ile, leu, tyr, phe and lys). The effect of age on the mean relative content of amino acids for which the interactions between genotype and age were significant, is shown for Com, GL and FC birds in figure 2a, 2b and 2c respectively. In most cases, the interaction was caused by a age-difference for Com males, whereas this age-effect was nearly absent in the other genotypes. For those amino acids, where no interaction between genotype and age was present, the effect of genotype is shown in figure 3. For cys, ser, pro and val the difference observed was a

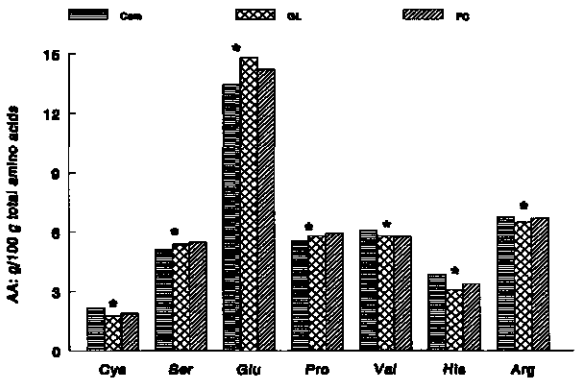

Figure 3. Amino acid pattern in different genotypes. Mean for 14 and 49 days old birds for those amino acids of which no significant interaction between genotype and age was found. * denotes significant contrasts ($P<0.05$).

difference between Com genotype and both experimental lines (FC and GL). For glu, all three genotypes were significantly ($P<0.001$) different from each other. For his and arg the value of FC genotype was more close to the Com genotype than that of GL genotype.

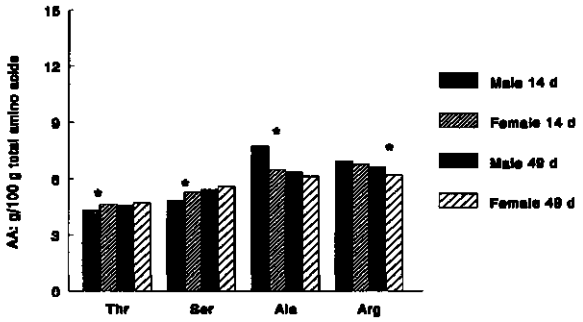

Figure 4. Amino acid pattern of male and female broiler chicks at 14 and 49 days of age. Amino Acids with significant (F-test; $P<0.05$) interaction. * denotes significant contrasts ($P<0.05$).

Table 5 shows the amino acid pattern of male and female chicks of Com genotype at both 14 and 49 days of age. Between sex and age a significant interaction ($P<0.05$) was present only for four amino acids (thr, ser, ala and arg). As shown in figure 4, for thr, ser and ala this was a result of male 14 day old birds, differing from the other birds whereas for arg it resulted from 49 day old female birds differing from the others. For the amino acids where the interaction of sex and age was not significant, the mean effect of sex is shown in figure 5. Female chicks have a lower cys, met, val, tyr, phe and his content than males whereas asp, glu, ile and leu were higher in females.

Discussion

Age

The change in amino acid pattern with age was studied most extensively for male chicks of the Com genotype.[1] Changes in relative amino acid content were statistically significant, but in general small. Therefore, the nutritional relevance of significant differences could be a point of discussion. For this reason only

[1] These data have also been published in a preliminary form (ten Doeschate et al., 1991).

Table 3. Amino acid pattern in male broiler chicks, corn genotype. Amino acid content as a percentage of total analyzed amino acids (g/100 g). (n = 6 per subclass; except for day-old chicks n = 2)

	Age (d)									
	0	7	14	21	28	35	42	49	F-prob.	
AA									sed	P
Cys	2.9	2.23	1.88	1.92	2.13	2.40	2.49	2.43	0.096	***
Asp	9.0	9.04	8.83	8.82	9.03	9.14	8.68	8.92	0.104	**
Met	2.4	2.52	2.47	2.39	2.45	2.58	2.40	2.53	0.077	NS
Thr	4.4	4.39	4.29	4.27	4.47	4.53	4.40	4.52	0.036	***
Ser	6.0	5.08	4.79	4.96	5.12	5.27	5.41	5.32	0.080	***
Glu	12.8	13.0	13.1	13.1	13.4	13.3	13.0	13.3	0.111	*
Pro	4.9	5.05	5.35	5.84	5.43	5.76	6.04	5.50	0.221	**
Gly	7.9	7.88	8.31	8.51	7.20	7.01	8.11	6.89	0.212	***
Ala	6.3	6.62	7.66	7.57	6.93	6.55	6.79	6.34	0.171	***
Val	6.2	6.36	6.02	5.85	5.75	5.84	5.82	6.15	0.171	*
Ile	4.3	4.51	4.31	4.40	4.49	4.52	4.43	4.73	0.078	***
Leu	7.5	7.53	7.31	7.27	7.49	7.50	7.35	7.63	0.061	***
Tyr	3.4	3.37	3.15	3.15	3.26	3.17	3.11	3.22	0.063	**
Phe	4.8	4.38	4.18	4.22	4.31	4.36	4.33	4.35	0.059	*
His	3.2	3.46	3.66	3.53	3.95	3.77	3.66	4.02	0.084	***
Lys	6.1	6.72	6.75	6.53	6.88	6.68	6.32	6.74	0.087	***
Arg	7.0	6.90	6.88	6.70	6.72	6.62	6.77	6.48	0.066	***
Trp	0.94	0.97	1.01	0.95	1.04	1.03	0.90	0.89	0.034	***

F-probability: * < 0.05; ** < 0.01; *** < 0.001

Table 4. Amino acid pattern (g/100 g total analyzed amino acids) in broiler chicks of three genotypes (Com, GL and FC) at 14 and 49 days of age.

Age	14 days			49 days			F-probability		
Geno	Com	GL	FC	Com	GL	FC	Age	Geno	A*G
AA									
Cys	1.90^{bc}	1.54^{a}	1.73^{ab}	2.44^{d}	2.00^{c}	2.04^{c}	***	***	NS
Asp	8.91^{a}	9.45^{c}	9.20^{bc}	9.01^{ab}	9.11^{ab}	8.90^{a}	**	***	**
Met	2.49^{c}	2.37^{bc}	2.20^{ab}	2.53^{c}	2.11^{a}	1.97^{a}	*	***	NS
Thr	4.32^{a}	4.71^{b}	4.67^{b}	4.59^{b}	4.58^{b}	4.66^{b}	NS	***	***
Ser	4.82^{a}	5.08^{ab}	5.30^{bc}	5.40^{cd}	5.67^{e}	5.65^{de}	***	**	*
Glu	13.4^{a}	15.0^{c}	14.2^{b}	13.5^{a}	14.6^{bc}	14.2^{b}	NS	***	NS
Pro	5.44^{a}	5.49^{ab}	5.80^{ab}	5.65^{ab}	6.14^{b}	6.09^{b}	*	*	NS
Gly	8.38^{b}	7.33^{a}	7.80^{ab}	6.97^{a}	7.86^{ab}	7.84^{ab}	NS	NS	***
Ala	7.73^{b}	6.33^{a}	6.30^{a}	6.36^{a}	6.33^{a}	6.33^{a}	***	***	***
Val	6.07	5.70	5.71	6.07	5.89	5.80	NS	*	NS
Ile	4.37^{a}	4.76^{b}	4.72^{b}	4.76^{b}	4.80^{b}	4.73^{b}	**	***	**
Leu	7.39^{a}	8.19^{d}	7.97^{cd}	7.70^{b}	7.90^{bc}	7.80^{bc}	NS	***	***
Tyr	3.18^{b}	3.23^{b}	3.16^{b}	3.25^{b}	2.84^{a}	3.02^{ab}	***	NS	***
Phe	4.21^{ab}	4.20^{ab}	4.14^{a}	4.36^{b}	4.06^{a}	4.18^{ab}	NS	*	**
His	3.68^{cd}	2.94^{a}	3.35^{bc}	4.01^{d}	3.18^{ab}	3.46^{bc}	**	***	NS
Lys	6.80^{ab}	7.08^{b}	6.93^{b}	6.79^{ab}	6.47^{a}	6.68^{ab}	**	NS	*
Arg	6.93^{c}	6.58^{ab}	6.78^{bc}	6.62^{ab}	6.45^{a}	6.66^{ab}	***	**	NS

^{a,b,c} ; Different indices within a row indicate significant differences. (Two-sided t-test; $P<0.01$)

Com = commercial broiler hybrid; GL = line selected for high growth rate; FC = line selected for low feed conversion ratio; Geno = main effect of genotype; Age = main effect of age; A * G = interaction between age and genotype

F-probability: * < 0.05; ** < 0.01; *** < 0.001

Table 5. Amino acid pattern (g/100 g total analyzed amino acids) in Com broiler chicks of both sexes at 14 and 49 days of age.

Age	14 days		49 days		F-probability		
Sex	Male	Female	Male	Female	Age	Sex	A*S
Cys	1.90^{ab}	1.59^{a}	2.44^{c}	1.92^{b}	***	**	NS
Asp	8.91^{a}	9.38^{bc}	9.01^{ab}	9.44^{c}	NS	**	NS
Met	2.49^{b}	2.18^{a}	2.53^{b}	2.26^{ab}	NS	**	NS
Thr	4.32^{a}	4.63^{b}	4.59^{b}	4.70^{b}	***	**	**
Ser	4.82^{a}	5.29^{b}	5.40^{bc}	5.57^{c}	***	**	*
Glu	13.38^{a}	14.74^{b}	13.50^{a}	15.12^{b}	NS	***	NS
Pro	5.44	5.82	5.65	5.66	NS	NS	NS
Gly	8.38^{b}	8.19^{b}	6.97^{a}	6.90^{a}	***	NS	NS
Ala	7.73^{c}	6.50^{b}	6.36^{ab}	6.13^{a}	***	***	***
Val	6.07	5.69	6.07	6.02	NS	*	NS
Ile	4.36^{a}	4.53^{ab}	4.76^{bc}	4.91^{c}	***	*	NS
Leu	7.39^{a}	7.86^{bc}	7.70^{b}	8.03^{c}	**	***	NS
Tyr	3.18	3.07	3.25	2.96	NS	**	NS
Phe	4.21	4.07	4.36	4.16	NS	*	NS
His	3.68^{bc}	3.04^{a}	4.01^{c}	3.30^{ab}	*	***	NS
Lys	6.80	6.61	6.79	6.72	NS	**	NS
Arg	6.93^{c}	6.80^{bc}	6.62^{b}	6.19^{a}	***	*	**

^{a,b,c} : Different indices within a row indicate significant differences. (Two-sided t-test; $P<0.02$)
F-probability: * < 0.05; ** < 0.01; *** < 0.001
Age = main effect of age; Sex = main effect of sex; A * S = interaction between age and sex

differences of more than 10 % of the mean are considered further. As described in table 3, a relatively large difference was found for 4 amino acids between 21 and 28 days of age; for two amino acids between 7 and 14 and 25 and 42 days of age, whereas one such difference was found between 28 and 35 days of age and between 42 and 49 days of age. A t-test on the contrast of each consecutive sampling moment against the mean of the preceding sampling moments, resulted in 4 to 9 significant ($P<0.02$; average 6) out of the 18 possible contrasts. Therefore, when focusing on the amino acid pattern of body accretion, the data do not present conclusive evidence for a division of the growth period in several phases. There is, however, also a certain pattern observed in the effect of age on relative whole body amino acid content of broiler chicks. For several amino acids (9) the values during the first part of the growth period (7-21 days of age) are either low or high compared to the values in the second part (28-49 days of age. This shift in amino acid pattern occurs around 21 days of age. Based on the most important ($>10\%$) differences between sampling moments and the shift in pattern observed around 21 days of age, we propose to divide the growth period in two phases; the first from 0-21 days of age and the second from 22 days of age until slaughter (49 d).

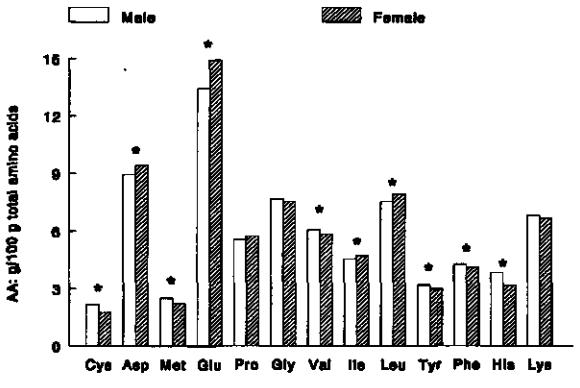

Figure 5. Amino acid pattern of male and female broiler chicks. Mean of 14 and 49 days of age for those amino acids of which no significant (F-test $P>0.05$) interaction was found between sex and age. * denotes significant contrasts ($P<0.05$).

In practical feeding, often a three-phase feeding system is employed. The reason for this approach is that approximately a week before slaughter the diet should be changed because coccidiostats, routinely used, require a withdrawal period to comply with existing regulations. In practice, this gives an additional opportunity to adapt the diet more closely to the birds requirements. A commonly used practical three phase system consists of a starter diet for 0-14 days of age, a grower diet from 15 days of age on and a finisher diet for the last week before slaughter. To provide amino acid patterns of whole body accretion to be used in both a two and a three phase feeding system, the amino acid content

of the analyzed birds were combined with growth data from each respective growth phase. The resulting amino acid patterns, with lysine as a reference amino acid, are given in table 6. According to Baker and Han (1994) the dietary ratios of amino acids (true digestible basis, with lysine = 100) in the early growth phase of chicks are: met + cys 72 %; thr, 67 %; val, 77%; arg, 105 %; his, 32 %; ile, 67 %; trp, 16 %; leu, 109 %; phe + tyr, 105 %; gly + ser, 65 % and pro, 44 %. The amino acid pattern of body accretion in our experiment differs in some cases somewhat from the ratios proposed by Baker and Han (1994). In body accretion cys, arg and trp were slightly lower. The non-essential amino acids pro and gly (+ser) were considerable higher in the profile for body accretion than the requirement ratios proposed by Baker and Han (1994). The essential amino acid his was also considerable higher in body accretion than in the requirement ratio. In ongoing studies (ten Doeschate et al., to be published) we will test the resulting nitrogen efficiency and meat production, when diets based on the pattern of Baker and Han (1993) and based on the amino acid pattern of whole body accretion, are compared.

A comparison of the whole body amino acid patterns of broiler chicks, as affected by age, determined in this study with those patterns described in the literature, is shown in table 7. Compared to the older patterns, cys, met, val phe and his are higher in the modern broiler whereas pro, gly and ser are somewhat lower. The much higher content of his in the present study is remarkable.
The amino acid pattern found in our study suggests that the modern broiler contains relatively more muscle (val, phe) and feathers (met,cys) and less collagen (gly, pro) than the birds of which compositions were reported in the literature.

Genotype

Differences in growth and feed conversion ratio between GL and FC line were comparable to those reported by Leenstra & Pit (1987). The similarity in performance between GL and Com genotype, indicates that for selection of Com genotype probably growth has also been a very important criterium. Parallel curve analysis of growth, fitted against age (weeks) for all genotypes, revealed that for FC genotype the best fit was obtained by a straight line whereas for GL and Com genotype addition of an exponential part to a straight line resulted in a better fit. Thus the growth rate of the FC genotype increased linearly with time, whereas for the other genotypes the increase of growth rate slowed down with age. This difference in growth pattern might explain the observed difference in overall feed

conversion efficiency. During the first weeks of age, efficiency of FC birds was similar to that of other genotypes while during week 3 to 7 efficiency of FC birds was distinctly better. The selection method used for selection of FC-birds thus has resulted in a chick that grows very efficient in the period 21-49 days of age due to a constant increase in growth rate during this period. The results of the growth study suggest that differences between GL and Com genotype are of minor importance, whereas the FC genotype differs clearly from the other two genotypes. We therefore expected also concomitant differences in amino acid pattern between the three genotypes. Thus if genotype would have an effect on whole body amino acid composition, there would be a contrast between Com and GL genotype on the one hand and FC genotype on the other. The starting population of both experimental lines was based on sire lines of commercial broiler hybrids. So differences between commercial and experimental genotypes can be considered to be differences due to the selection process and not due to differences in initial genetic background. 10 out of 17 amino acids displayed an interaction between genotype and age (Table 4). As shown in figure 2, this interaction was in most cases caused by absence of an age-effect in both experimental lines, while results of the Com-genotype were affected by age. The whole body amino acid composition of Com birds thus changes more with age than that of GL or FC birds. For the amino acids without interaction, the three genotypes can be compared directly. In contrast to our expectation, the effect of genotype was not found in a contrast between FC and the combination of Com and GL. The contrast was mainly between both experimental lines and Com genotype, where often the FC-line was more close to Com genotype than was the GL-line. The low amount of cys in whole body of GL birds may be explained by the observation that, especially young, GL birds have less feathers than Com or FC birds. Some non-essential amino acids (ser, glu, pro) were higher in the experimental lines whereas some essential amino acids (cys, val, his and arg) were lower. This indicates a marked difference between the commercial broiler and experimental lines. Present commercial broilers will have thus a higher requirement for some essential amino acids than both experimental lines.

The effect of genotype on amino acid pattern can not be explained by the observed differences in growth pattern. This implies, that for a correct assessment of amino acid pattern of a given genotype, it is necessary to determine the actual amino acid content, since birds with a similar growth pattern such as Com and GL genotype, may still differ in amino acid pattern. For the assessment of the whole body amino acid content, it might be important to analyze separate body components such as organs, feathers and muscle. Thus, amino acid requirements

Table 6. Mean amino acid pattern of body accretion during the growth period; divided in two or three growth phases. Amino acids expressed relative to lysine = 100.

	Two-phase system		Three-phase system		
Period (d)	0-21	22-49	0-14	15-35	36-49
Cys	28	38	25	33	40
Asp	134	132	129	132	134
Met	36	38	36	35	39
Thr	65	68	62	66	69
Ser	75	80	67	76	83
Glu	201	197	193	195	201
Pro	90	79	79	79	84
Gly	131	94	122	98	103
Ala	117	87	115	96	91
Val	89	92	88	83	99
Ile	67	71	63	66	74
Leu	111	114	106	110	117
Tyr	48	48	46	48	49
Phe	64	65	60	63	67
His	54	61	54	59	61
Lys	100	100	100	100	100
Arg	102	94	100	96	96
Trp	15	13	15	15	12

Table 7. Whole body amino acid pattern (g/100 g total amino acids) as presented in different sources.

Source[1]	1		2	3			4	5		
Age(d)	14	42	14	14	21	35	28	14	35	49
W (g)	146	863	80-90	333	620	1318	250-600	340	1697	2830
Cys	1.78	1.88	1.99	2.02	2.06	2.41	2.14	1.88	2.40	2.43
Asp	9.21	8.18	8.73	9.08	8.90	8.42	8.88	8.83	9.14	8.92
Met	2.25	1.79	1.91	1.79	2.17	1.86	2.14	2.47	2.58	2.53
Thr	4.48	4.18	4.03	4.47	4.56	4.24	4.39	4.29	4.53	4.52
Ser	5.18	5.38	4.41	5.79	5.37	6.37	5.39	4.79	5.27	5.32
Glu	13.4	13.5	14.5	13.6	12.6	12.4	13.6	13.1	13.3	13.3
Pro	6.70	7.95	6.47	7.29	8.03	7.32	6.84	5.35	5.76	5.50
Gly	8.19	8.86	?	8.85	8.69	8.64	8.88	8.31	7.01	6.89
Ala	6.44	6.31	7.11	6.5	6.41	6.23	6.33	7.66	6.55	6.34
Val	5.79	6.38	5.83	4.44	4.42	4.21	5.13	6.02	5.84	6.15
Ile	4.61	4.70	4.80	3.75	3.52	3.78	4.41	4.31	4.52	4.73
Leu	8.03	7.37	7.75	7.73	7.49	7.43	7.45	7.31	7.50	7.63
Tyr	3.65	3.05	3.22	3.36	3.26	3.39	2.86	3.15	3.17	3.22
Phe	4.01	4.18	4.78	4.15	4.02	4.37	4.18	4.18	4.36	4.35
His	2.33	2.55	2.25	2.80	2.50	3.39	3.27	3.66	3.77	4.02
Lys	6.53	6.16	6.04	6.84	8.36	7.65	6.53	6.75	6.68	6.74
Arg	6.11	6.31	7.26	6.84	6.95	7.22	6.94	6.88	6.62	6.48

[1] *References:* 1 = Holmes et al., 1963; 2 = Saunders et al., 1977; 3 = Kreuzer et al., 1988; 4 = Kirchgessner et al., 1988; 5 = present investigation

of birds will change according to differences in body amino acid pattern as selection continues. Different selection goals or methods might result in birds with different amino acid requirements for optimal performance. In an ideal situation, breeding companies should therefore provide amino acid requirements of the different types/lines.

Sex

Sex and age showed little interaction (table 5 and figures 4 and 5). For thr, ser and ala, the observed interaction was caused by differences between sexes in 14 day old chicks, whereas this effect was absent in 49 day old birds. For arg, the difference between sexes was absent in 14 day old birds whereas at 49 days of age female birds had a lower relative arg content than male birds. For most amino acids the effects of age and sex were thus independent which implies that female and male chicks in essence showed similar shifts in amino acid pattern with age. In case of a significant effect of sex, the amino acids with lower values in female chicks were all essential amino acids. Effects of sex on amino acid pattern could be expected to result from differences in feathers and in muscle content. Especially in Com birds, with a sex-linked gene for slow feathering, female birds were expected to have more feathers than male birds (Lowe & Merkley, 1986). Thus it is surprising that female chicks had relatively less cys and met than male Com birds. Possibly the slower feathering of male birds only affects total plumage early in development, without a lower cys content at 14 and 49 days of age. The amino acids with lower contents in female birds are all considered to be essential to the bird. Female chicks might thus have a lower requirement (lysine = 100) for some essential amino acids than male chicks of the same genotype. In general, significant differences between male and female chicks were less than 10 % of the mean value. Only for cys, met, glu and his a larger difference was found.

General validity of established amino acid pattern for diet formulation

It is clear (table 3, 4 and 5), that the whole body amino acid pattern of broiler chicks is influenced by age, genotype and sex. The patterns shown in table 6 can be used as basis for diet formulation in a two or three phase feeding system. However, it should be noted that minor adjustments to these patterns might be necessary, to account for non-productive amino acid requirements such as

maintenance or inevitable losses due to inefficiency (Schreurs et al, 1994). The general question is: is it always necessary to use exactly the correct pattern for birds of a given age, genotype and or sex or might it be possible to use a more general amino acid pattern ? In the European Amino Acid Table (Janssen et al., 1992) a summary of more than 75,000 amino acid analyses of more than 100 foodstuffs is given. From this large data set, it can be concluded that most foodstuffs have a highly variable amino acid content (or alternatively that amino acid analyses, used for composition of that table, gave highly variable results). For solvent extracted soybean meal or fish meal two times the standard deviation of the amino acids relative to nitrogen was, respectively, 7 and 11 % of the mean. Based on this high variability in amino acid content of foodstuffs we conclude that, in practice, it will be impossible to compose diets matching exactly the differences in body amino acid patterns caused by differences in genotype/sex or age. Most differences in whole body amino acid pattern were approximately 10 % of the mean, which is about similar to the variability found in the foodstuffs. An alternative would be to determine amino acid contents in each batch of foodstuffs. However, this is hardly feasible nor affordable in practice. Based on these considerations we consider, at this stage, that it is not relevant to use different amino acid patterns for birds of different genotypes or sexes. Since it is common to use two or three diets during the growth period of broilers, we propose to use in those periods the proper amino acid patterns of body accretion of male Com birds as the basis for the dietary amino acid pattern. The Com genotype is chosen, because this chick is more representative for the modern broiler than both experimental lines. We chose to use male chicks because female chicks have a lower content of some essential amino acids. Utilisation of a 'female' pattern for male chicks would result in undersupply for some essential amino acids and would thus probably impair growth and lower nitrogen efficiency whereas utilisation of a diet with a 'male' pattern for female chicks will only lead to a small oversupply of some essential amino acids without major negative side-effects.

Conclusions

Whole body amino acid pattern of broiler chicks, expressed as a percentage of total analyzed amino acids, is affected by age, genotype and sex. Birds with similar growth pattern (Com and GL) can differ in whole body amino acid composition. Complete adjustment of dietary amino acid composition to whole body amino acid composition of each chick (genotype/sex) with time is neither

feasible nor necessary. For adjustment of the dietary amino acid pattern to the development of the bird, it is proposed to use either a two-phase or a practical three phase feeding system. In all cases, it is proposed to use the amino acid pattern of body accretion of male chicks of Com genotype as the basis for dietary amino acid composition during the respective growth phases.

Comparison of the amino acid pattern determined in the chicks of Com genotype with literature values revealed, that the amino acid pattern of the modern broiler differs from birds investigated in the past. These differences suggest, that in a modern broiler the proportion of muscle has increased at the expense of collagenous tissue.

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Chapter 2

DIGESTIBILITY STUDIES IN BROILER CHICKENS: INFLUENCE OF GENOTYPE, AGE, SEX, AND METHOD OF DETERMINATION

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Summary

1. The influences of genotype, age and sex on droppings digestibility coefficients of a compound food were studied using male and female broiler chickens of three different genotypes at 2, 4 and 6 weeks of age.
2. Because the traditional method of determination of droppings digestibility coefficients of nitrogen may lead to systematic errors in estimating the feeding value of foodstuffs, a method is proposed to determine the ileal digestibility coefficients. The ileal method is compared with the droppings method for a mixed food and for two foodstuffs: wheat and solvent-extracted soyabean meal.
3. Birds selected for efficient food conversion showed distinctly higher digestibility coefficients for all nutrients than birds selected for high growth potential or birds from a commercial strain.
4. The influence of age on digestibility coefficients was not consistent.
5. Female birds showed digestibility coefficients which were, in general, 3% higher than those of male chickens.
6. Interactions of genotype and sex and between genotype and age for energy metabolisability were the only interactions observed for digestibility measurements.
7. The method of determination influenced the amino acid digestibility coefficients of the mixed food and the relative feeding values of wheat and soyabean meal.
8. It is important to use well defined animals (genotype, sex, age) in evaluating foodstuffs.
9. The preferred method for determination of digestibility coefficients of nitrogen and amino acids is based on ileal sampling, although the differences in amino acid digestibility coefficients were small between methods.

Introduction

Digestibility of food ingredients for broilers may depend on genotype, age and sex of the animals. For reliable application of digestibility coefficients in practice, it is necessary to quantify these effects. Influences of genotype on digestion have been reported (Sørensen et al., 1983; Leenstra and Pit, 1988; Jørgensen et al., 1990). With respect to the influence of age, findings are equivocal. In some studies an age influence was absent (McNab and Shannon, 1972; Chwalibog et al., 1978; Sørensen et al., 1983), while in others an increase (Haakansson and Eriksson, 1974; Wallis and Balnave, 1984) or a decrease (Haakansson et al., 1978) of metabolilisability of energy or digestibility of the organic matter with age was found. The reason for these differences is not always clear. Protein digestibility seems to decrease with age (Haakansson and Eriksson, 1984), while amino acid digestibility increases (Wallis and Balnave, 1984). This might be explained by the difference between analytical protein (6.25 x nitrogen) on the one hand and amino acids, the constituents of protein on the other hand. No influence of sex on digestibility was found (Chwalibog et al., 1978; Sørensen et al., 1983; Wallis and Balnave, 1984; Leenstra and Pit, 1988; Gruhn and Zander, 1989).

In recent years, protein digestion studies in pigs have focused on the estimation of ileal digestibility, because this is considered to be a better estimate of protein availability than faecal digestibility. In poultry this is not the case because the influence of microbial processes in the avian caeca and colon on digestibility is considered to be less important. For nitrogen and nitrogenous substances, however, not only are microbial processes important, but contamination of digesta with urine also constitutes a problem, because in birds faeces and urine are excreted together. The content of uric acid in droppins can be determined chemically (Terpstra and de Hart, 1974), but this method still remains questionable. Because uptake of nitrogenous substances is thought to take place before or in the ileum (Webb,1990) it can be argued that digestibility should be measured at the terminal ileum to get the best estimate of availability. A method was, therefore, developed in which ileal digestibility was determined by means of a slaughter technique as an adaptation of the method used by van der Klis et al. (1990). Similar techniques have been described for pigs by Moughan and Smith (1987), and for poultry by Summers and Robblee (1985).

In this paper three experiments are described. In the first experiment, the effects of genotype, age and sex on the excreta digestibility coefficients of dry matter, nitrogen and amino acids, and on the apparent metabolisability of energy were determined.

In the second experiment, a comparison was made between excreta digestibility determined by a sampling method and ileal digestibility determined by a method developed in our institute. In this experiment, the same broiler diet was used as in experiment 1. In the third experiment, this comparison was repeated using different diets. The purpose of the third experiment was to determine whether the relative feeding values of wheat and soyabean meals were influenced by the method of determination.

Material and methods

Environment

All chickens were housed in battery cages with a wire floor (surface area 0.45 m^2). The environmental temperature was degreased gradually from 32°C for one-d-old chickens to 18°C at six weeks of age. For the first 3 d, there was continious light. To prevent leg disorders, the birds were kept under a lighting regime in 1 h light alternating 2 h darkness from 3 d of age. Continuous food intake is desirable to establish a steady state in the intestinal tract of the chickens (experiments 2 and 3) as was described by van der Klis et al. (1990). To enable continuous light food intake throughout the day, the lighting regime was restored for the remaining birds (experiment 1). The birds had *ad libidum* access to food and water.

Statistics

Statistical analysis was done by analysis of variance, using Genstat (Genstat 5 Committee, 1987). The models used are given for each experiment separately. Effects were tested for significance using an *F*-test. Differences between treatments were tested by *t*-test. Results are given as mean values and standard errors of difference (S.E.D.). Significance is shown by use of the following symbols: ns = not significant: + = $P<0.10$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

Experiment 1

Male and female chickens of three broiler strains were used. One line had been selected for efficient food conversion between 21 and 42 d of age (FC line). The second

one had been selected for high body weight at 42 d of age (GL line). Chickens were from the 11th generation of the selection experiment described by Leenstra and Pit (1987). The third line (Com) was a commercial broiler hybrid (Euribrid, Boxmeer). Food composition for experiment 1 is given in Table 1. Food intake was recorded and the birds were weighed at 0, 21, 35 and 41 d of age. At approximately 2, 4 and 6 weeks (13 to 15, 27 to 29 and 41 to 43 d) of age digestibility trials were carried out. In each trial, food intake and droppings production were measured quantitatively per cage over 3 consecutive days. In each cage there were 20, 13 and 9 birds at 2, 4 and 6 weeks, respectively. Droppings were stored at -18°C immediately after daily collection. After thawing, droppings were homogenised, refrozen, freeze dried and ground to pass a 1 mm seive.

The available cages were distributed over 4 blocks so that a blocked design could be used. Within the blocks one replicate of each genotype/sex combination was assigned at random to a cage.

Table 1. Food composition (g/kg)

	Experiments 1 + 2	Experiment 3, basal diet
Maize	359.4	691.1
Soyabean meal (solvent extracted)	237.0	200.0
Wheat	119.0	...
Tapioca	77.0	...
Soyabeans (toasted)	47.0	...
Animal fat	40.0	...
Soya oil	30.0	45.0
Fish meal	23.0	...
Feathermeal	20.0	...
Sunflower meal (solvent extracted)	13.0	...
L-Lysine-hydrochloride	...	1.5
DL-Methionine	2.8	1.6
Minerals + vitamins	31.0*	60.0**
Narasin (cocciodiostat)	0.5	0.5
Zinc bacitracin	0.3	0.3

* Minerals and vitamins added according to NRC requirements (NRC, 1984)

** Minerals and vitamins added at twice NRC requirements, because the basal diet was mixed with foodstuffs on a equal basis.

Apparent metabolisme energy content (AME) of the diet was calculated as:

$$AME = (E_{food} - E_{droppings} - c \times N_{retained})/DM_{intake}$$

where: AME = apparent metabolisable energy content (MJ/kg DM); E_{food} = gross food energy intake (MJ/d); $E_{droppings}$ = gross droppings energy loss (MJ/d); c = correction factor for zero N-balance = 0.3653 (MJ/gN); $N_{retained}$ = amount of nitrogen retained (g/d); DM_{intake} = food dry matter intake (kg/d). Metabolisability was calculated by dividing the AME content of the food by its gross energy content.

Model 1 was used to determine the influences of genotype, age and sex on growth, food conversion ratio (FCR) and the metabolisability and digestibility coefficients of dry matter (dDM) and nitrogen (dN):

$$Y = \mu + \text{block} + \text{age} + \text{genotype} + \text{sex} + \text{age x genotype} + \text{genotype x sex} + \text{age x genotype x sex} + e \quad \{\text{Model 1}\}$$

The influence of age on amino acid digestibility coefficients was tested for male Com chickens only, according to Model 2:

$$Y = \mu + \text{block} + \text{age} + e \quad \{\text{Model 2}\}$$

At 4 weeks of age the effects of genotype and sex on amino acid digestibility coefficients were determined with Model 3:

$$Y = \mu + \text{block} + \text{genotype} + \text{sex} + \text{genotype x sex} + e \quad \{\text{Model 3}\}$$

Experiment 2

In this experiment, male Com chickens were given the same food as used in experiment 1. To allow digestibility to be determined 1 g/kg chromic oxide was included as a non-absorbable marker. The pre-experimental feeding period started at day 22. At day 27, droppings were collected twice for 2-h periods. Sample preparation was similar to experiment 1. At day 28 the chickens were killed by an intravenous injection of T-61 [a mixture of embutramide, mebezonium iodide and tetracain hydrochloride (0.2, 0.05 and 0.005 g/ml, respectively), Hoechst, Munchen]. Immediately after death the abdomen was opened and the ileum was exposed. Two 5 cm segments of the posterior part of the ileum were ligated. The last 2 cm anterior to the ileo-caecal sphincter were not sampled, to avoid contamination with urine. The anterior segment

was called ileum 1 (Il 1) and the posterior segment ileum 2 (Il 2). The two parts were removed, and the segments were emptied between thumb and finger with gentle pressure to prevent damage to the intestinal mucosa. The whole procedure took about 2 min per chicken. Chyme samples were pooled for all animals in a cage. After freeze-drying, the ileal
samples were ground. In this experiment 8 replicate cages (15 birds/cage) were used, evenly distributed over 4 blocks.

Because digestibility measurements were based on a marker, digestibilities of the nutrients in the food were calculated as follows:

$$DC_{food} = 1\text{-}[(M_{food}/M_{d,c}) \times (C_{d,c}/C_{food})]$$

where: DC_{food} = digestibility coefficient of a nutrient in the food; M_{food} = marker concentration in food; $M_{d,c}$ = marker concentration in droppings (d) or chyme (c); C_{food} = concentration of nutrient in food; $C_{d,c}$ = concentration of nutrient in droppings (d) or chyme (c). The effect of determination method on digestibility coefficients was tested using Model 4:

$$Y = \mu + \text{block} + \text{cage} + \text{method} + e \qquad \{\text{Model 4}\}$$

Experiment 3

The experimental procedure was similar to that followed in experiment 2. However, in this experiment, three different diets were used. The first was the basal diet (Table 1). The other two were prepared by mixing equal amounts of basal diet and either wheat (wheat diet) or solvent extracted soyabean meal (soyabean meal diet). For this experiment 4 replicate cages per diet were used distributed over 4 blocks.

The digestibilities of the nutrients in the foodstuffs in experiment 3 were calculated as follows:

$$DC_{fs} = (DC_{diet} \times C_{diet} - DC_{basal} \times C_{basal} \times 0.5)/(C_{diet} - C_{basal} \times 0.5)$$

where: DC_{fs} = digestibility coefficient foodstuff: DC_{basal} = digestibility coefficient basal diet; C_{diet} = concentration of nutrient in diet; C_{basal} = concentration of nutrient in basal diet.

The effects of foodstuff, determination method and interaction on the digestibility coefficients were tested using Model 5:

$$Y = \mu + \text{block} + \text{foodstuff} + \text{method} + \text{foodstuffxmethod} + e \qquad \{\text{Model 5}\}$$

Analysis

Nitrogen contents of food, droppings and chyme were determined by a standard Kjehldahl procedure. Nitrogen in droppings was corrected for uric acid according to the method of Terpstra and de Hart (1974). Chromium was analysed with an atomic absorption spectrophotometer. Amino acid concentrations were determined with a LC-ion exchange column after reaction with ninhydrin. Norleucine was used as an internal standard. Values for Ser, Thr, Ile and Val were corrected according to Slump (1969). For all determinations of amino acid digestibility using droppings, it should be realised that Gly digestibility will be underestimated, because Gly is formed from uric acid even during oxidative hydrolysis (Slump et al., 1977). Nevertheless, the results are presented, because the magnitude of the problem may differ with the factors under investigation. Energy contents were determined by total combustion in an adiabatic bomb calorimeter.

Results

Experiment 1

The main effects of genotype and sex on growth and food conversion ratio are given in Table 2.

There were no interactions between genotype and sex. The significant effect of genotype was mainly the result of differences between the FC line on the one hand and Com and GL line on the other. Female chickens showed a lower growth rate and a higher food conversion rate than male chickens. Table 3 shows the effect of genotype, age and sex on the digestibilities of dry matter (DM) and nitrogen (N), as well as on the metabolisability of gross energy. The FC line showed a significantly higher DM digestibility than the other two genotypes. Chickens of 27 to 29 d of age had a lower DM-digestibility than younger and older birds. Sex had no significant influence on DM digestibility.

Table 2. Weight gain (g/d) and food conversion ratio (FCR, g food/g weight gain) of different genotypes and sexes.

	0 to 21 d		21 to 35 d		35 to 41 d	
	Growth	FCR	Growth	FCR	Growth	FCR
Genotype						
Com	32.0	1.41	59.3	1.82	67.0	2.01
GL	34.0	1.41	63.8	1.83	73.2	2.01
FC	23.3	1.35	53.2	1.63	62.3	1.75
SED	0.56	0.016	1.2	0.019	5.2	0.11
P	***	**	***	***	ns	*
Sex						
male	30.8	1.37	62.2	1.72	73.8	1.83
female	28.7	1.41	55.3	1.8	61.2	2.01
SED	0.45	o.o13	1.0	0.015	4.2	0.089
P	***	*	***	**	**	+

Com = commercial broiler hybrid; GL = growth selected line; FC = food conversion selected line. SED = standard error of a difference. P: ns = not significant: + = $P<0.10$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

All genotypes differed significantly in N digestibility. Birds of the FC-line had the highest value, and birds of the GL line the lowest. N digestibility was not consequently affected by age; the second period showed the lowest N digestibility, while the first period showed the highest value. Female birds showed a greater N digestibility than males. No interactions between genotype, age or sex were found for digestibilities of N and DM.

The metabolisability of energy in FC was higher than for Com or GL. Chickens of six weeks of age obtained more AME from the diet than did younger chickens. For metabolisability of energy, there were interactions between genotype and age ($P<0.01$) and between genotype and sex ($P<0.05$). In the GL line male and female chickens did not differ, while a sex effect on metabolisability was significant for the other two genotypes (Fig. 1). At 13 to 15 d of age, the Com genotype showed a lower metabolisability of energy than the other lines (Fig. 2). In general, the Com genotype showed a metabolisability value between that of FC and GL line.

Table 4 shows the food intake during each digestibility trial, expressed as g/kg metabolic body weight (body weight raised to the power of 0.75). Body weight was an estimate, based on the weight measured closest to the digestibility trial and the average growth rate in the respective period. Because of the relatively low growth rate of young birds in the first week, body weight in the first trial will be slightly overestimated, because weight was recorded at 21 d of age and the digestibility trial was performed at 13-15 d of age. Relative food intake in this period was, therefore, probably underestimated.

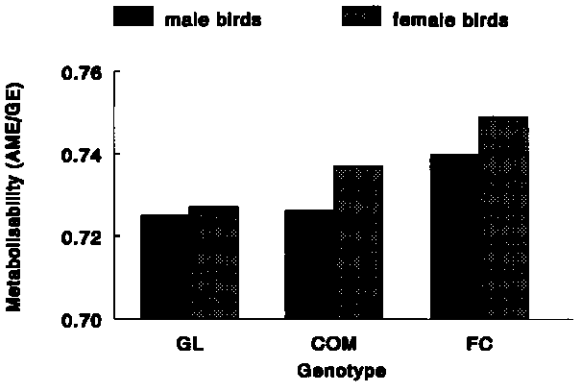

Figure 1. Interactions between genotype and sex for metabolisability (apparent metabolisable energy (AME) content divided by gross energy (GE) content). GL = growth selected line; FC = food conversion selected line; Com = commercial broiler hybrid. (SED = 0.0023.)

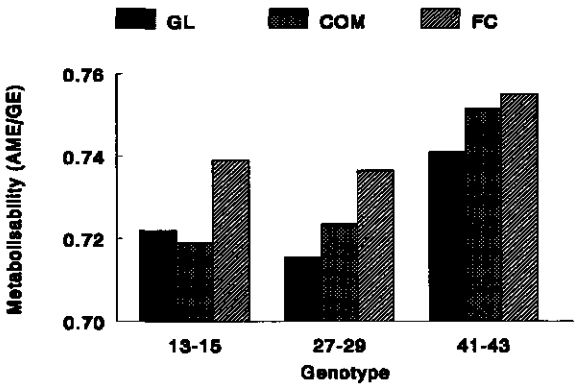

Figure 2. Interactions between genotype and age for metabolisability (apparent metabolisable energy (AME) content divided by gross energy (GE) content). GL = growth selected line; FC = food conversion selected line; Com = commercial broiler hybrid. (SED = 0.0028.)

Table 4 shows that food intake relative to metabolic weight was influenced by age and genotype. Interactions between age and genotype and between age and sex were also present. Interactions between age and genotype were found, resulting from the relatively low food intake of the GL line in the first period. An interaction between age and sex was found in the second period, in which male chickens had a higher food intake than female chickens, whereas in other periods sex differences did not exist.

Table 3. Effect of genotype, age and sex, on digestibility coefficients (DC) of dry matter and nitrogen and on metabolisability of gross energy

	DC dry matter	DC nitrogen	Metabolisability of gross energy
Genotype			
Com	0.711	0.848	0.731
GL	0.702	0.839	0.726
FC	0.733	0.867	0.744
SED	0.0019	0.0027	0.0016
P	***	***	***
Age (d)			
13 to 15	0.720	0.860	0.727
27 to 29	0.707	0.842	0.725
41 to 43	0.720	0.851	0.749
SED	0.0019	0.0027	0.0016
P	***	***	***
Sex			
Male	0.714	0.846	0.730
Female	0.716	0.857	0.737
SED	0.0016	0.0022	0.0013
P	ns	***	***

Com = commercial broiler hybrid; GL = growth selected line; FC = food conversion selected line. SED = standard error of a difference. P: ns = not significant: + = $P<0.10$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

Table 5 shows the effect of age on amino acid digestibility for male Com chickens. Nearly all amino acids were better digested during the third period than during the first two periods. Nitrogen, however, was best digested in the first period. The comparisons among genotypes and sexes were made at 4 weeks of age for amino acid digestibility; these results are given in Table 6. It is clear that an effect of genotype and sex on digestibility coefficients existed at 27 to 29 d of age for nearly all amino acids and total nitrogen and dry matter. There were no interactions between genotype and sex. The FC line showed an increase in digestibility coefficients for all nutrients

over the Com genotype of about 3% . The slight differences in digestibility coefficients between GL and Com line were not statistically significant.

Table 4. Food intake during each digestibility trial as influenced by genotype, sex and age. Expressed as g dry matter/kg metabolic body weight.

Age (d):	13 to 15 d	27 to 29 d	41 to 43 d	Main effects
Genotype				
Com	85.4	86.5	84.6	85.5
GL	72.8	92.0	85.7	83.5
FC	77.6	82.0	73.7	77.8
SED		1.87		1.08
P		***		***
Sex				
Male	77.3	88.9	81.4	82.5
Female	79.9	84.8	81.3	82.0
SED		1.53		0.88
P		*		ns
Main effect age	78.6	86.9	81.3	
SED		1.08		
P		***		

Com = commercial broiler hybrid; GL = growth selected line; FC = food conversion selected line. SED = standard error of a difference. P: ns = not significant: + = $P<0.10$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

Table 5. Effect of age on digestibility coeffeicients at droppings level of amino acids and nitrogen

Age (d)	13 to 15	27 to 29	41 to 43	Mean	(SED)	*P*
Digestibility coefficients of:						
cys	0.633	0.649	0.717	0.666	(0.0105)	*
asp	0.788	0.800	0.839	0.809	(0.0041)	*
met	0.895	0.898	0.907	0.900	(0.0042)	ns
thr	0.742	0.750	0.800	0.764	(0.0056)	*
ser	0.791	0.798	0.842	0.810	(0.0044)	*
glu	0.865	0.866	0.895	0.875	(0.0042)	*
pro	0.853	0.844	0.888	0.862	(0.0072)	*
gly	0.549	0.479	0.425	0.484	(0.0812)	ns
ala	0.779	0.776	0.817	0.791	(0.0081)	*
val	0.780	0.784	0.814	0.793	(0.0053)	*
ile	0.829	0.831	0.859	0.840	(0.0045)	*
leu	0.832	0.837	0.862	0.844	(0.0049)	*
tyr	0.838	0.843	0.869	0.850	(0.0083)	*
phe	0.833	0.837	0.865	0.845	(0.0044)	*
his	0.780	0.793	0.828	0.801	(0.0126)	*
lys	0.833	0.838	0.857	0.843	(0.0037)	*
arg	0.886	0.887	0.898	0.890	(0.0027)	*
nitrogen	0.849	0.830	0.840	0.840	(0.0048)	*

P: ns = not significant: + = $P<0.10$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

Table 6. Effect of genotype and sex on digestibility coefficients at droppings level of amino acids, nitrogen and dry matter at 27-29 d of age

Digestibility coefficients of:	Genotype					Sex			
	Com	GL	FC	SED	*P*	male	female	SED	*P*
Digestibility coefficients of:									
cys	0.658	0.635	0.700	0.0117	***	0.656	0.675	0.0095	*
asp	0.811	0.801	0.843	0.0051	***	0.811	0.826	0.0042	**
met	0.910	0.898	0.922	0.0054	**	0.905	0.915	0.0044	*
thr	0.764	0.753	0.797	0.0055	***	0.762	0.781	0.0045	***
ser	0.810	0.801	0.843	0.0045	***	0.811	0.827	0.0037	***
glu	0.873	0.870	0.894	0.0032	***	0.875	0.884	0.0026	**
pro	0.856	0.859	0.878	0.0079	*	0.863	0.866	0.0065	ns
gly	0.431	0.412	0.491	0.0369	ns	0.493	0.404	0.030	*
ala	0.787	0.781	0.815	0.0052	***	0.787	0.802	0.0042	**
val	0.794	0.785	0.823	0.0046	***	0.793	0.809	0.0037	***
ile	0.842	0.837	0.868	0.0038	***	0.841	0.857	0.0031	***
leu	0.847	0.841	0.875	0.0042	***	0.847	0.863	0.0034	***
tyr	0.852	0.847	0.877	0.0046	***	0.854	0.864	0.0037	*
phe	0.846	0.841	0.872	0.0037	***	0.847	0.860	0.0030	***
his	0.795	0.798	0.805	0.0054	ns	0.797	0.802	0.0044	ns
lys	0.845	0.842	0.863	0.0032	***	0.845	0.855	0.0026	**
arg	0.893	0.886	0,912	0.0026	***	0.893	0.901	0.0021	**
N	0.839	0.827	0.862	0.0033	***	0.835	0.849	0.0027	***
DM	0.702	0.692	0.725	0.0034.	***	0.704	0.710	0.0028	*

Com = commercial broiler hybrid; GL = growth selected line; FC = food conversion selected line. SED = standard error of a difference. P: ns = not significant: + = $P<0.10$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

Table 7. Comparison of digestibility coefficients determined at two ileal levels (IL 1, IL 2) and at droppings level in 4-week-old commercial type broiler chicks

Level:	IL 1	IL 2	droppings	SED	*P*
Digestibility coefficients of:					
cys	0.610	0.597	0.686	0.0174	***
asp	0.798	0.793	0.843	0.0065	***
met	0.936	0.927	0.922	0.0053	+
thr	0.777	0.771	0.805	0.0067	***
ser	0.808	0.802	0.837	0.0058	***
glu	0.885	0.879	0.891	0.0040	*
pro	0.913	0.915	0.882	0.0069	***
gly	0.790	0.781	0.765	0.0138	ns
ala	0.847	0.845	0.832	0.0054	*
val	0.828	0.830	0.831	0.0053	ns
ile	0.859	0.859	0.866	0.0049	ns
leu	0.862	0.856	0.872	0.0055	*
tyr	0.860	0.860	0.886	0.0076	**
phe	0.864	0.863	0.872	0.0049	ns
his	0.834	0.833	0.831	0.0087	ns
lys	0.871	0.871	0.877	0.0053	ns
arg	0.889	0.888	0.908	0.0038	***
N	0.804	0.802	0.857	0.0074	***
DM	0.718	0.709	0.709	0.0075	ns

P: ns = not significant: + = $P<0.10$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

Experiment 2

Digestibility coefficients for nitrogen, dry matter and amino acids determined by different methods are given in Table 7. Differences between determination methods were found in the comparison of ileal and droppings DC. Between the two segments of the ileum there was an indication ($P<0.10$) of a difference only for met DC. For all other amino acids no significant differences were found between the two ileal segments. Six amino acids (cys, asp, thr, ser, tyr and arg) showed a droppings digestibility coefficient (DC) distinctly higher than the ileal DC. For Pro and Ala, DC determined at the ileum were higher than those determined from the droppings. Nitrogen showed a higher DC in the droppings than in the ileal chyme. This means that the difference between ileal and droppings DC for total nitrogen was not equal to those of the individual amino acids, nor that of the average of the amino acids.

Experiment 3

Table 8 shows the DC of the wheat and solvent-extracted soyabean meal calculated by correcting the DC of the experimental diets for the DC of the basal diet. The DC of soyabean meal was also expressed as a percentage of the DC of wheat. For nearly all nutrients, the ranking of the foodstuffs was essentially the same regardless of the method used. Only the differences between wheat and soyabean meal differed with the method used.

Discussion

Genotype

Differences between FC and GL lines for growth and FCR were similar to those described by Leenstra and Pit (1987). The results for Com were comparable to those for GL. In commercial broiler selection, growth potential has probably been the most important trait. Also for the most digestion traits the influence of genotype can be considered as a difference between FC on the one hand and GL and Com on the other. The results of the present experiment for AME content and N digestibility are consistent

Table 8. Digestibility coefficients (DC) of wheat (W) and soyabean meal (SBM); comparison between determination methods. % = 100*(DCSBM/DCW)

	ileal 1			ileal 2			droppings			SED	SED
	W	SBM	%	W	SBM	%	W	SBM	%	method	food
DC of:											
cys	0.853	0.783	92	0.780	0.701	90	0.814	0.727	89	0.030	0.025
asp	0.720	0.785	109	0.728	0.800	110	0.743	0.850	114	0.014	0.026
met	0.845	0.881	104	0.809	0.870	108	0.811	0.901	111	0.022	0.026
thr	0.641	0.770	120	0.666	0.787	118	0.699	0.793	113	0.017	0.042
ser	0.790	0.808	102	0.802	0.821	102	0.792	0.848	107	0.017	0.018
glu	0.919	0.823	90	0.915	0.824	90	0.925	0.854	92	0.0088	0.010
pro	0.896	0.864	96	0.929	0.863	93	0.911	0.810	89	0.017	0.006
gly	0.745	0.771	104	0.750	0.780	104	0.647	0.483	75	0.048	0.009
ala	0.672	0.801	119	0.674	0.812	121	0.665	0.805	121	0.024	0.034
val	0.764	0.806	105	0.776	0.824	106	0.775	0.825	106	0.014	0.011
ile	0.823	0.822	100	0.824	0.834	101	0.826	0.852	103	0.019	0.011
leu	0.799	0.811	102	0.754	0.808	107	0.815	0.840	103	0.024	0.018
tyr	0.785	0.834	106	0.809	0.860	106	0.897	0.899	100	0.0063	0.015
phe	0.832	0.822	99	0.865	0.843	97	0.897	0.855	95	0.0083	0.016
his	0.758	0.821	108	0.768	0.834	109	0.809	0.834	103	0.019	0.022
lys	0.734	0.869	118	0.726	0.878	121	0.771	0.891	116	0.021	0.035
arg	0.818	0.865	106	0.828	0.873	106	0.838	0.915	109	0.016	0.014
N	0.808	0.792	98	0.829	0.811	98	0.831	0.876	105	0.012	0.010
DM	0.698	0.510	73	0.711	0.554	78	0.758	0.372	49	0.014	0.010

with the results found by Leenstra and Pit (1988).

The genetic differences in the present experiment (chickens of the 11th generation) were found to be larger than those found in chickens of the 7th generation (Leenstra and Pit, 1988). The differences in metabolisability, N digestibility and amino acid digestibility related to genotype were similar to differences in dry matter digestibility. This means that the genotypes did not show a preferential digestion of nitrogen or any other nutrient. In the FC line overall digestion was improved compared with the

GL line. The better digestibility shown by the FC line was paralleled by a lower relative food intake. This lower food intake might lead to a lower passage rate and consequently improved digestibility, as suggested by Cherry and Siegel (1978).

Age

If any effect of age were to be found one would expect that metabolisability would increase with age (e.g. Wallis and Balnave, 1984). In this experiment, only during the last period was there any indication that apparent metabolisability increased. For DM and N digestibilities, when comparing the first and second period, a decrease can be seen in accordance with the findings of Haakansson and Eriksson (1974). The third period gave similar results to the first period. The amino acid digestibility was influenced by age in the same manner as the metabolisability of the energy. After the second experimental period it was necessary to treat an infection by adding Tetrafur (tetracycline + furaltadone) to the drinking water. It might, therefore, be possible that the results of the third period were influenced by the antibiotics (e.g. Shafey and McDonald, 1991) or those of the second period by a subclinical infection.

Dry matter and nitrogen digestibility were related to food intake in the same way as was found for the genotype effect. However, amino acid digestibility and metabolisability were not affected in this way by differences in food intake. It can be concluded that the way age influences digestibility is still not entirely clear, but that an influence of age on digestibility does exist.

The fact that the influence of age on N digestibility was not the same as on the digestibility of most amino acids (Table 5) means that nitrogen does not describe true protein digestion accurately. This might be explained by the method of faecal nitrogen determination (Terpstra and de Hart, 1974; Terpstra, 1979). In this method faecal nitrogen is determined by chemical determination of uric acid and other nitrogenous compounds in the excreta. Normally, this method gives quite acceptable results (Terpstra and de Hart, 1974). However, it is also known that an over- or underestimation of the true faecal nitrogen content may occur. When using amino acid digestibility the contamination of faeces with urinary nitrogen is a problem only for gly. If urinary nitrogen excretion is not constant then differences between amino acid digestibility and nitrogen digestibility are to be expected. Because older chickens will have a relatively higher excretion, an underestimation of N digestibility at greater ages is likely. The decrease with age in determined gly digestibility, although not statistically significant, also points in this direction.

Sex

Significant effects of sex on digestibility coefficients were found. This contrasts with earlier work (Chwalibog et al., 1978; Sørensen et al., 1983; Wallis and Balnave, 1984; Leenstra and Pit, 1988; Gruhn and Zander, 1989). Female broiler chickens showed a slightly better metabolisability, N digestibility and amino acid digestibility. These higher digestibility measurements were related, however, to a higher food conversion ratio. Apparently, the efficiency with which digested food was used for growth was lower in female birds. The differences in digestibility measurements were small (about 3%) and always in the same direction. If the assumption is made that energy intake determines food intake, it is not necessary to formulate different foods for male and female chickens to take account of differences in digestion. Female chickens digested all nutrients more effeciently, so the relative intake of metabolisable energy and digestibler nitrogenous compounds is equal for male and female chickens. When performing digestibility experiments, it is therefore necessary to use animals of the same sex in order to prevent over- or underestimation of feeding values.

Interactions

For the production parameters growth and food conversion ratio no interactions were found. It is interesting that, for metabolisability of energy, interactions between genotype and sex and between genotype and age did exist. GL male and female chickens showed a similar metabolisability while the females of the other genotypes in general had higher metabolisability values. However, this different response in metabolisability was not reflected in food conversion ratio. In the first period, the Com line gave the lowest metabolisability while in the other two periods the Com line had figures intermediate between the GL and FC lines. This interaction also was not reflected in food conversion ratio.

Food intake expressed as g dry matter per kg metabolic body weight showed interactions between genotype and age and between sex and age. In the first period, the GL line had the lowest calculated food intake while in other periods the GL line had the highest food intake. Because the GL line had a higher growth rate it is possible that body weight estimation for the GL line in the first period was less accurate than for the other two genetic groupes and that this may have resulted in statistical interaction. Sex differences in food intake relative to metabolic weight existed only in the second period, which might have been the result of higher food intake for male

chickens in this period caused by very rapid growth. For nitrogen and amino acid digestibilities, no interactions were found between genotype, age and sex.

Method of determination

The contamination of faeces with urine in chickens and the consequences for determination of N digestibility have prompted the development of an ileal method. There were differences between ileal and droppings digestion coefficients, while differences between two successive 5 cm parts of the terminal ileum (IL1 and IL2) could not be found. Summers and Robblee (1985) found no differences in digestibility coefficients in three successive 6 cm parts of the terminal third of the ileum. Therefore, it is concluded that the use of a length of up to 10 cm (as we did in our experiment) is justified. However, the closer the sampling site is to the terminal ileum the more accurate is the estimate of total amino acid digestibility, because amino acid absorption is considered to continue throughout the ileum (Webb, 1990). When differences between IL2 and droppings were found, the droppings DC was usually higher than the ileal DC. This means that amino acids have disappeared during passage from ileum to droppings. Uptake of amino acids and other nitrogenous compounds is not thought to take place after the terminal ileum (Webb, 1990). These amino acids may have disappeared by some other route, for instance by microbial fermentation. This implies that droppings digestibility will overestimate the availability of those amino acids. For Pro the opposite was found. Although the lower droppings digestibility coefficient of Pro can possibly be ascribed to the microbial formation of Pro from other amino acids, we realise that other explanations are possible. Differences between droppings and ileal digestibility are not very large for the food used. For other foods differences might be larger. In less well balanced foods excess amino acids will lead to a higher urinary N excretion, while in less digestible foods microbial fermentation will be stimulated.

Experiment 3 showed that the relative feeding value of two foodstuffs (wheat and soyabean meal) was influenced by the method of determination of the digestibilty coefficients. However, the ranking of both foodstuffs is the same regardless of the method used. The very low apparent dry matter digestibility for soyabean meal at droppings level can be explained by the high uric acid excretion necessary to eliminate the excess nitrogen uptake from the soyabean meal diet. From the chemical formula of uric acid it can be calculated that excretion of 1 g excess nitrogen as uric acid produces an excretion of 3 g dry matter. The fact that apparent dry matter digestibility

is higher in IL 2 than in IL 1, based on mixed samples from 15 birds, can be seen as proof that contamination with urine from the cloaca is negligible.

From this paper it can be concluded that it is necessary to determine digestibility of foodstuffs in animals of approximately the same age and genotype as those to which the given foodstuff will be fed. With regard to sex, it is necessary to standardise digestibility experiments so animals of the same sex are used. In our experiments we chose to use male broiler chickens of approximately 4 weeks of age. The determination method should be based on theoretically correct assumptions. We have shown that ileal digestibility leads to a different relative feeding value from digestibility based on droppings. Uric acid contamination and microbial fermentation are considered problems in the droppings determination. The use of the ileal technique described is, therefore, more reliable.

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Chapter 3

$^{14}CO_2$ BREATH TEST MEASUREMENTS IN BROILER CHICKS: RELEVANCE OF ROUTE OF TRACER INJECTION AND TIME OF INJECTION RELATIVE TO A MEAL.

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Abstract

1. The effect of nutritional status (dietary lysine content, time of injection relative to the meal) on $^{14}CO_2$ recovery from intraperitoneal (ip) injected [U-^{14}C]lysine was studied in broiler chicks.
2. It was found that ip injection of [^{14}C]labelled amino acids ([U-^{14}C]lysine) sometimes resulted in unrealistic high recovery values in $^{14}CO_2$ breath test measurements. The most probable explanation is that with ip-injection in some cases label is injected in an abdominal air sac. In this case part of the substrate is not converted to $^{14}CO_2$ but is straight excreted via an aerosol. For this reason ip-injection of tracer amino acids is considered to be risky in chickens and therefore subcutaneous injection is used in further experiments.
3. When only the lower range of $^{14}CO_2$ recoveries from [U-^{14}C]lysine were considered, lysine content of the diet caused a response in $^{14}CO_2$ recovery while time of injection did not give an detectable effect. Low lysine diet resulted in lower $^{14}CO_2$ recovery, indicating a restriction of lysine oxidation.
4. In birds conditioned on a lighting regime of 1L:2D, $^{14}CO_2$ recovery from subcutaneous injected [1-^{14}C]leucine or [1-^{14}C]valine was studied within one cycle of the lighting regime with injections at 30, 60, 120 or 180 minutes after onset of a half-hour meal.
5. For both [1-^{14}C]leucine and [1-^{14}C]valine $^{14}CO_2$ recovery was higher when injection at 30 or 60 min after onset of a half hour meal is compared to injection at 120 or 180 min.
6. The rate of $^{14}CO_2$-release, based on a Gompertz fit of the curve of $^{14}CO_2$-recovery against time, was lower when injection of tracer is given at 180 min after onset of the meal compared to 30 or 60 min.

Introduction

The principle of $^{14}CO_2$ recovery after application of a [^{14}C]substrate has often been used in metabolic studies (eg. Colvin et al, 1969, Ball & Bayley, 1984, Bergner et al., 1987, Simonnet et al., 1989). In our laboratory Schreurs et al. (1992) and Weijs (1993) initiated the $^{14}CO_2$ breath test measurement for their studies on amino acid metabolism in rats. This $^{14}CO_2$ breath test measures the cumulative recovery of label in the breath 4 hour after a single shot intraperitoneal (ip) injection of a [^{14}C]amino acid as substrate. A similar setting was also used for $^{14}CO_2$ breath test measurements in broiler chicks to measure the effect of nutritional status on amino acid oxidation. As described in this paper, the intraperitoneal injection in broilers may, however, cause problems. Especially in chickens, relevance of route of tracer injection on $^{14}CO_2$ recovery is not well known. Tracer solutions are in some studies injected intravenously (Bergner et al., 1987) while in other studies chickens were injected intraperitoneally (Simonnet et al., 1989). With intravenous injection the exact dose injected is hard to determine while in intraperitoneal injected birds the effective injection site is not certain. Subcutaneous injection might be a valid alternative. In the present paper is shown that ip-injection can cause problems in $^{14}CO_2$ breath test whereas with sc-injection no problems were encountered.
The effect of nutritional status of the birds is poorly investigated in broiler chicks. Nutritional status of the birds, eg time of injection relative to the meal, differs between studies. For broilers, no studies are known on different injection times relative to the meal in relation to $^{14}CO_2$ production. In the present investigation, relevance of nutritional status of the bird in relation to $^{14}CO_2$ recovery is discussed.

Materials and methods

Respiration units

The design of the $^{14}CO_2$ collection device used for chickens was based on the units as used for rats in our laboratory (Schreurs et al., 1992). Since individually housed birds behave disturbed we equipped the respiration units for use by two chicks.

Birds were housed in a darkened pvc respiration chamber (40*40*45 cm). The incoming air was dried with spoca[1] to facilitate evaporative heat loss of the birds. On the bottom of the chamber a wired floor was placed to avoid contact between chicks and excreta. Outlet air from the cage was drawn with a suction pump (± 8 l/min) through a sintered (G4) glass filter just above the bottom of a cylindrical (Ø 12 cm) sample flask. Since only the solution above the filter is sampled, before starting the experiment the fluid underneath the filter is drawn through the filter. The pressure gradient over the filter, maintained by the suction pump, prevents the fluid from dripping back. The sample flask contained circa 1-1.5 l 2 M KOH, depending on the weight of the birds. The exact amount is determined by weighing. Temperature inside the respiration chambers was monitored and, if necessary, ambient temperature was adjusted (by heating or ventilation) to keep inside temperature close to the chicks normal environmental temperature.

Birds and housing

Broiler chicks (Hybro: Euribrid, Boxmeer) were housed in groups on battery cages with a wired floor. The environmental temperature was decreased gradually from 33°C for one-day old chicks to 18°C at 6 weeks of age. During the first 3 d there was continuous light. Thereafter, to prevent leg disorders, the birds were kept under a lighting regime of 1 h light alternating with 2 h darkness. This lighting regime also promotes a regular pattern of feed intake and thus a relative continuous flow of nutrients. Because the chicks are accustomed to a regular alternation of dark and light periods, stress as a result of the placement in the dark respiration chambers during the $^{14}CO_2$ breath test will be reduced.

Diets and feeding

The amino acid composition of the control diets (A, Table 1) was based on the amino acid pattern of body accretion in the respective growth phases (ten Doeschate et al., 1991). In exp. 1 only the diet for the first growth phase (0-3 wk

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1) Sponge drenched with saturated $CaCl_2$-solution and dried. Capable of drying inlet air up to 20 % relative humidity.

of age) was used. Diet B was prepared by exchange of lysine-HCl by maize (reduction of 33 % in total lys).

Table 1. Feed composition diet A in phase 1 and 2 (0-21 and 22-43 days of age respectively; phase 2 only used in exp. 2).

	Phase 1 g/kg	Phase 2 g/kg
Wheat	248.66	246.1
Maize	216.35	174.6
Maize starch	-	98.5
Maizeglutenfeed	102.3	93.1
Lupins, white	85.1	24.7
Sunflower seed, extracted, decort.	80.0	18.2
Meat meal	70.0	-
Soya bean oil	75.0	73.8
Soya beans, extracted	48.0	155.6
Maizeglutenmeal	38.5	36.2
Sugar	-	29.5
Minerals*	16.2	29.75
Vitamins*	5.0	5.0
Coccidiostat	0.5	0.5
Zinkbacitracin	0.3	0.3
lysin-HCl	5.0	4.43
DL-methionine	2.83	2.09
glycine	3.8	3.19
L-histidine	1.3	2.29
L-threonine	0.65	0.80
L-valine	0.51	1.35
Total	1000	1000

* Minerals and vitamins were added to comply with NRC standards.

Calculated nutrients:		
Apparent Metabolisable Energy (MJ/kg)	12.4	12.6
Crude protein (g/kg)	213	178
Lysine (g/kg)	11.9	10.7
Methionine + cysteine (g/kg)	8.9	7.4
Methionine (g/kg)	5.8	4.6

Starting from day 0, diet A was fed *ad libitum* until the pre-experimental day, when birds were housed with two together and the experimental diet (A or B) was fed. On the experimental day, feed was removed at the end of a light period early in the morning. After 2 h, when the lights went on, birds were allowed a 30 min test meal.

Injection of tracer

Injection of tracer substances, ± 37 KBq (1 μCi) in 200 μl aqueous solution, was either given intraperitoneally (ip, exp. 1) or subcutaneously (sc, recovery + exp. 2). The bird was placed on the back, legs held cranial, wings spread. For ip-injection, the needle was inserted approximately 1 cm from the pubic bones, aimed to pass through the abdominal fat pad and without stirring internal organs. Sc-injection was given in a fold of the skin at the lateral side of the body just cranial to the right leg. The injected dose is determined by weighing the syringe plus needle before and after injection.

Measurement of ^{14}C-output

In the breath, $^{14}CO_2$ recovery is essentially measured according to Schreurs et al. (1992). Immediately after injection with [^{14}C]amino acid, two birds were placed in a respiration chamber, the chambers were sealed with adhesive tape and ventilation was started. All exhaust air was drawn through the sample flask for a period of 4 (ip) or 4.5 (sc) hours after injection. During the initial 60 and the final 30 minutes of the measurement period every 15 minutes a 2 ml sample was taken from the flask. In between, samples were taken every 30 minutes. At the end of the experiment, birds were killed by adding diethylether (*Exp. 1*) or a mixture of 70% CO_2 and 30 % O_2 (*Exp. 2*) to the respiration chambers.

[$^{14}CO_2$] in the samples was determined by counting a 1.0 ml subsample mixed with 10 ml liquid scintillation cocktail (Ultima Gold, Packard) in a Philips scintillation counter (LSC4700).

In the first experiment excreta were collected to get an impression of faecal label loss. Radioactivity in faeces was counted as advised (Cocktail guide, Canberra Packard, 1989). Before routine use, the technique was calibrated by addition of [1-^{14}C]leucine as an internal standard.

Some samples of the excreta contained high levels of radioactivity. On average 3.6 % (sd 2.97) of the injected dose was recovered in excreta. No effect of time of injection or diet on recovery of ^{14}C in excreta was apparent. Also recovery of ^{14}C in excreta was not correlated with $^{14}CO_2$ recovery. For this reason excreta were not sampled in experiment 2.

Calculation and statistics

The cumulative amount of radioactivity recovered from the breath was expressed as percentage of the injected dose. Corrections were made for the decreasing volume of KOH in the sample flask due to the removal of the samples (Schreurs et al., 1992). Figure 1 might serve as a typical example of the shape of the curve of expired $^{14}CO_2$ as a percentage of injected dose. Generally this curve displayed, after an initial lag phase, a phase of exponential increase ending in a plateau reached at about 4-4.5 hours after injection. The average of the last three values of the curve was taken as plateau value and thus as recovery of label.

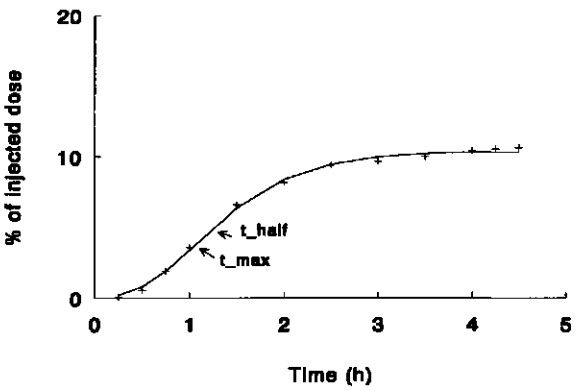

Figure 1. Example of a $^{14}CO_2$ recovery curve after sc-injection of [1-^{14}C]valine at 60 min after a half-hour meal of a normal diet (A).
+ = Results of a measurement of two 22 d old chicks (799+877 g BW)
— = Fitted Gompertz curve (According to formula 1, see text)
t_{half} is calculated from the parameters determining the fitted Gompertz curve according to formula 2, see text.
t_{max} is the parameter from the fitted Gompertz curve which determines the point where $^{14}CO_2$ recovery rate is maximal.

The data were analyzed using Genstat (Genstat 5 committee, 1987) as a statistical program. At first homogeneity of variances was checked for all data by Bartlett's and Hartley's test (Seber, 1977; Digby et al.,1989). If one of these tests showed that variances were inhomogeneous between groups then analysis was performed using group variances given by Genstat to calculate SED for the two sided t-test. In all other cases normal ANOVA was performed. In general a randomized block design was used with the following model:

$$Y_{it_1t_2h} = \mu + block_i + trea_{t_1} + trea_{t_2} + interactions + e_{it_1t_2h}$$ where

Y = responsevariable, μ = mean, block = week of age, $trea_{t_1,t_2}$ = treatment(s) applied, interactions = possible interactions between treatments.

The data of *exp. 2* were used to fit a Gompertz equation to the curve of recovery of $^{14}CO_2$. The generalized form of a Gompertz equation is:

$$y = \frac{c}{e^{e^{-b*(t-m)}}} \qquad \text{[Formula 1]}$$

Where y = % recovery at time t; c = estimated asymptotic end value; m = timepoint where recovery rate is maximal (t_{max}); b = constant for each curve. For each measurement a Gompertz fit was performed (Genstat 5 committee, 1987) after which the estimated time of maximal $^{14}CO_2$ recovery rate (t_{max}) and the time needed to reach half value (t_{half}) were analyzed with ANOVA. t_{half} was calculated as:

$$t_{half} = m - \frac{\ln(-\ln(\frac{1}{2}))}{b} \qquad \text{[Formula 2]}$$

$^{14}CO_2$ Recovery

To validate the $^{14}CO_2$-recovery procedure an experiment was performed with one bird per unit in four respiration units. Birds were sc-injected with approximately 37 KBq (1 μCi) $NaH^{14}CO_3$ (Amersham) each, placed in a respiration chamber and $^{14}CO_2$ was collected for 4.5 h. Since, probably due to exchange with atmospheric $^{12}CO_2$ (Hoerr et al., 1989), a solution of pure $NaH^{14}CO_3$ shows a diminishing concentration of radioactivity, the $NaH^{14}CO_3$-solution was diluted 1:100 with normal $NaHCO_3$.

Exp. 1

Oxidation of [U-^{14}C]lysine (Amersham) was measured after ip-injection at 30, 60, 120 or 180 minutes after onset of a meal of diet A or B. In two consecutive weeks (blocks, 12-15 and 19-22 d of age) four replicates per time/diet combination were performed.

Exp. 2

Oxidation of [1-^{14}C]leucine (Amersham) or [1-^{14}C]valine (Amersham) was measured after sc-injection at 30, 60, 120 or 180 minutes after onset of a meal of

diet A. During the second and fifth week of age (blocks), for each amino acid/time combination four replicate measurements were made.

Experimental results and discussion

Recovery

Within 4.5 h after injection of 37 KBq (1 μCi) $NaH^{14}CO_3$, diluted 1:100 with normal $NaHCO_3$, 90 % (sd = 1.8) was recovered in the breath. This recovery is in line with reported values (Reeds, 1974: > 98 %; Saunderson, 1985 > 97 %; Beckett et al., 1992: 83.3 %; Kim et al., 1983 > 98 %). Based on this result the respiration units and the method of $^{14}CO_2$ collection can be considered appropriate.

Route of injection of tracer

In an initial pilot (ten Doeschate et al., 1992), for a limited number of birds a comparison was made for the time course of $^{14}CO_2$ recovery after intraperitoneal (ip) or subcutaneous (sc) injection. Compared with ip-injection, after sc-injection a phase difference in the oxidation curve of approximately 30 minutes was apparent. This was probably caused by a slower absorption of tracer into the blood (ten Doeschate et al., 1992).

In experiment 1 the oxidation of [U-^{14}C]lysine was measured after ip-injection at four different time-points after a meal of a normal (A) or a lysine deficient (B) diet. The objective of the experiment was to study whether the use of the oxidation of [U-^{14}C]lysine as an indicator for the lysine supply, as described by Bergner et al. (1987), was affected by the time of injection relative to the meal. Sometimes unrealistic high recovery values were found. The distribution of data is shown in figure 2.

Results could be separated into three groups, one large group with low recoveries (0.5-5.5 %), one with high recoveries (>35 %) and an intermediate group. We concluded that our method did not give unequivocal results in birds. Based on literature values (Wang et al., 1973; Newport et al., 1976; Ball & Bayley, 1984; Bergner et al., 1987: zero to 10 % oxidation for low to normal lysine diets) the high figures are supposed to be artifacts. We hypothesize that in the intermediate group

one of both chicks in a respiration unit gave a high ^{14}C-recovery whereas in the high group both chicks gave this unphysiological response. In the low group both chicks in a unit responded normal. For that reason values of the low recovery group are summarized below:

Diet A (n = 22): Mean oxidation level = 3.5 %; sd = 1.23

Diet B (n = 24): Mean oxidation level = 1.0 %; sd = 0.43

Data indicate some difference in level of oxidation between diets. This result is in line with literature (Bergner et al., 1987), that in a lysine deficient diet lysine oxidation is lower than in a normal diet. The effect of injection time on $^{14}CO_2$ recovery from [U-^{14}C]lysine could not be tested due to the variability caused by the ip-injection. It is concluded that the supply of lysine relative to the requirement influences [U-^{14}C]lysine oxidation. However, due to the low level of oxidation differences in nutritional status (dietary lysine content, time of injection relative to the meal) are probably better studied by an indicator amino acid such as [1-^{14}C]leucine or [1-^{14}C]valine.

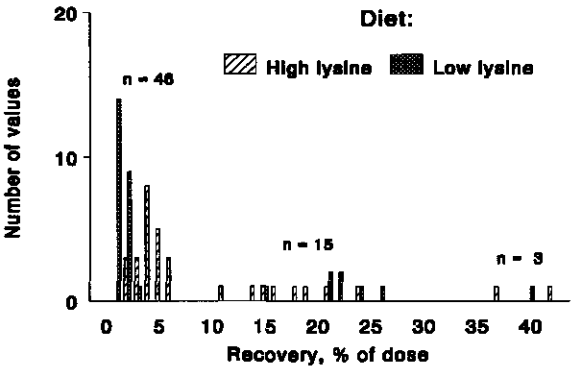

Figure 2. Distribution of $^{14}CO_2$ recovery measured in birds ip-injected with 37 KBq (1 μCi) [U-^{14}C]lysine.

The high recoveries observed sometimes with intraperitoneal injection suggest that ip-injection of the label sometimes favours unphysiological recovery of label. In our opinion an injection site which leads to unphysiological high recovery values should have the following characteristics: slow exchange of label with systemic circulation but easy exit of label to the breath. Before the experiment, in 10 birds was checked whether after ip-injection with a dye the dye would be found inside internal organs or the gut. After euthanasia the abdomen was opened. The dye was spread over the abdominal cavity without damage to abdominal organs. After experiment 1 was performed, we examined 19 additional dye-injected birds. Through careful examination, dye was found inside the abdominal air sac. It was shown that injection of dye into the bronchus of a dead bird results in a distinct colorization of the abdominal cavity, similar to the distribution of dye seen in some of the ip-injected birds. Since the wall of an air sac

has only limited blood supply (King, 1975), and the air sacs have a function in breathing, an abdominal air sac has the characteristics to favour unphysiological high recovery values as mentioned above. Injection into an air sac might even lead to direct excretion of [U-^{14}C]lysine in the air as an aerosol. This may be the most probable explanation for the high recovery found.

We conclude that there is serious doubt about the correctness and reproducibility of recovery measured with ip-injection in chicks. For this reason we changed to subcutaneous injection of the tracer for following experiments.

Nutritional status (Time of injection relative to meal)

Weijs (1993) has shown in rats that immediately after a meal oxidation of branched chain amino acids rises. This rise can be influenced by the amino acid content of the meal. The results of *exp. 2*, in which the effect of time of injection on oxidation of [1-^{14}C]leucine or [1-^{14}C]valine was measured, are shown in table 2.

Table 2. Recovery of $^{14}CO_2$ from [1-^{14}C]leucine and [1-^{14}C]valine as influenced by time of injection relative to a meal of a normal diet (A). (Mean (sd), n=4, different superscripts on a row indicate significant ($P<0.05$) differences)

		Time of injection after onset of a 30 min meal (min)			
Amino acid	Age (d)	30	60	120	180
[1-^{14}C]leu	11-14	19.5^{a} (0.35)	20.4^{a} (1.16)	16.2^{ab} (3.09)	12.1^{b} (1.55)
[1-^{14}C]leu	32-35	15.7^{a} (3.14)	13.1^{a} (1.97)	10.7^{a} (3.41)	10.5^{a} (2.51)
[1-^{14}C]val	11-14	12.5^{a} (1.82)	11.9^{a} (0.67)	12.2^{a} (1.89)	10.1^{a} (0.61)
[1-^{14}C]val	32-35	10.6^{a} (2.26)	10.8^{a} (0.59)	8.2^{ab} (1.52)	6.8^{b} (0.45)

From these results it can be seen that, although not always significant, injection time relative to the meal affected $^{14}CO_2$ recovery. No difference could be detected between injection 30 (end of the meal) and 60 minutes after onset of the half-hour meal. Injection 120 or 180 minutes after onset of the meal generally leads to lower recoveries. The majority of $^{14}CO_2$ from ^{14}C-labelled amino acids is excreted in the first hour after injection (Simonnet et al., 1989; Schreurs et al., 1992). The metabolic status of the bird in the first hour of the measurement will thus have a dominant influence on the final result. It should be recognized that injection at three hours after onset of the meal is equivalent to the time when they would otherwise

have taken their next meal since birds were used to a lighting regime of 1L:2D. In these birds a steady state inside the gut is not present (Van der Klis et al., 1990). Because of ceasing absorption of nutrients at 3 h after onset of the meal, the metabolism of the bird will start to react to a diminishing availability of nutrients. Oxidation will shift from a normal fed state to a fasted state which will result in a lower amino acid oxidation level.

Table 3. Parameters of curves fitted according to a Gompertz equation for the recovery of $^{14}CO_2$ from [1-^{14}C]leucine and valine, as influenced by time of injection. (Mean; n = 4; different superscripts on a row indicate significant differences)

	Time of injection (min) after onset of a 30 min meal of diet A (normal diet)				sed
Parameter	30	60	120	180	
t_{max} (h) leu	0.847^a	0.847^a	0.830^a	1.036^b	0.0541
t_{half} (h) leu	1.048^a	1.048^a	1.036^a	1.298^b	0.0645
$t_{max}{}^1$ (h) val	0.702^a	0.872^b	0.833^b	0.855^b	0.0590
$t_{half}{}^2$ (h) val	0.888^a	1.079^b	1.035^b	1.078^b	0.0642

During week 2 (11-14 d of age) there was a significant ($P<0.01$ for [1-^{14}C]leucine and $P<0.05$ for [1-^{14}C]valine) effect of time of injection relative to the meal on the parameters describing the shape of the recovery-curve (t_{max} and t_{half}). For chicks of 11-14 d of age these parameters are given in table 3. At 5 weeks (32-35 d) of age no differences were apparent (not shown). Both t_{max} and t_{half} indicate that the longer the time between meal and injection, the lower is the slope of the curve.

Our data show that the time of injection close to the meal, 30 or 60 minutes after onset of the meal, only resulted in differences in variation within treatments (Table 2). Injection after 60 minutes resulted in lower and more constant variation than injection after 30 minutes. Because of the lighting regime birds are expected to eat during the light period of each three hour period. At 30 minutes after onset of the meal some birds may have eaten just prior to the injection and some birds may have eaten in the beginning of the meal, while at 60 min, all birds had stopped eating at least 30 min ago. The physiological condition between birds will thus be more similar at 60 min. Furthermore, we expect that approximately 60 minutes after onset of the meal metabolic availability of dietary amino acids will be higher than at 30 minutes after onset of the meal. Since we are interested in metabolic

use of dietary amino acids we concluded to use an injection time of 60 minutes after onset of a half-hour meal in further experiments.

Conclusions

Intraperitoneal injection of tracer amino acids in broiler chicks may lead to injection into abdominal air sacs resulting in unphysiological high recovery figures. This problem can be avoided by using subcutaneous injection.

Time of injection (120 or 180 min vs 30 or 60 min) relative to a half-hour meal, in birds conditioned on a 1L:2D light regimen, affects $^{14}CO_2$ recovery from [1-^{14}C]leucine and [1-^{14}C]valine. The injection time of 60 minutes after onset of the meal is preferred for the study of metabolic utilization of dietary amino acids because of the relative low variation in $^{14}CO_2$ recovery and the expected high availability of dietary amino acids during the measurement.

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Chapter 4

$^{14}CO_2$ BREATH TEST MEASUREMENTS IN BROILER CHICKS: EFFECT OF DIETARY AMINO ACID LEVELS ON $^{14}CO_2$ RECOVERY.

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To be submitted

Summary

The effect of dietary amino acid content and of length of adaptation period on $^{14}CO_2$ recovery from [^{14}C]amino acids was studied in broiler chick breath test measurements.
A lower (70% of normal) dietary lysine level increased $^{14}CO_2$ recovery from [1-^{14}C]leucine as well as from [1-^{14}C]valine during the first growth phase (5-22 d of age). In older chicks this effect was less pronounced. A reduced vs increased dietary methionine level (67 vs 133 % of normal) increased $^{14}CO_2$ recovery from [1-^{14}C]leucine only at about 4 weeks of age. At a low dietary lysine level $^{14}CO_2$ recovery from [U-^{14}C]lysine was lower than at a normal lysine level. Length of adaptation period to a low lysine diet (1, 2, 3 or 5 d) had no statistically significant effect on $^{14}CO_2$ recovery from [1-^{14}C]leucine.
The utilization of leucine and valine for protein synthesis, as indicated by the complement of the $^{14}CO_2$ recovery from [^{14}C]amino acids, is affected by limiting dietary amino acid level. The age-dependent effect of dietary methionine level suggests that the method is especially suited for assessment of specific requirements during specific stages of development. The absence of influence of length of adaptation period to a low lysine diet on $^{14}CO_2$ recovery from [^{14}C]leucine suggests that adaptation is either rapid (< 1 day) or not measured with this approach. It is concluded that $^{14}CO_2$ recovery from [^{14}C]amino acids indicates amino acid adequacy of the diet fed immediately preceeding (20h period) the breath test measurement.

Introduction

Amount and ratio of dietary amino acid supply of animals is an important issue to optimize protein input with regard to protein deposition. Various methods of determination of amino acid requirements have been developed such as measurement of protein deposition in dependance of amino acid supply. This requires long term studies. Recently short term methods such as measurement of oxidation of indicator amino acids have been developed. The measurement of $^{14}CO_2$ recovery from [^{14}C]amino acids can be used to determine the adequacy of amino acid supply. An inadequate supply of the first limiting amino acid will create an excess of other amino acids and they will consequently be oxidized. The percentage $^{14}CO_2$ recovery from [^{14}C]amino acids indicates amount of oxidation of these amino acids. [^{14}C]Amino acids, other than the amino acid(s) varied and limiting in the diet, can thus serve as indicator amino acids for amino acid adequacy. This principle, called the indicator method, has been used by Bayley and co-workers in pigs (e.g. Kim et al., 1983; Ball & Bayley, 1984). When a [^{14}C]isotopomer of the limiting amino acid is used as substrate then $^{14}CO_2$ recovery will rise if dietary supply of the amino acid is increased above requirement. This so-called auto-indicator method has been used for lysine in chicks by Bergner et al (1987) and Bergner & Mnilk (1990, 1993). In our laboratory a dual breath test approach was developed for rats (Schreurs et al., 1992) and methodically modified for broiler chicks (ten Doeschate et al, 1995).

The aim of the present study was to investigate the effect of dietary amino acid levels and of length of pre-experimental adaptation period on the recovery of $^{14}CO_2$ from [^{14}C]amino acids and on parameters describing speed of recovery (*Exp. 2*). Long term effect of dietary amino acid levels on growth and feed conversion rate of chicks was determined in *exp. 1*.

[1-^{14}C]leucine and [1-^{14}C]valine were tested as indicator amino acids for the adequacy of the supply of lysine or methionine (*exp. 2*). The influence of the length of the low-lysine pre-test adaptation period on $^{14}CO_2$ recovery from [1-^{14}C]leucine was investigated (*exp. 3*). Furthermore it was aimed to measure the effect of low- or normal lysine diets on $^{14}CO_2$ recovery from [U-^{14}C]lysine in birds adapted to a low lysine diet (*exp. 4*).

Materials and methods

Birds and housing

Day old male wing-banded chicks from a commercial broiler hybrid (Hybro: Euribrid, Boxmeer NL) were housed in groups of 15 birds in battery cages with a wired floor. The environmental temperature decreased gradually from 33°C for one-day old chicks to 18°C at 6 weeks of age. After 3 d of continuous light, birds were kept under a lighting regime of 1 hour light alternating with 2 hours darkness (1L:2D) to reduce skeletal disorders.

Diets and feeding

All birds were fed diet 1A (0-21 days of age) or 2A (22-43 days of age) until the experimental treatment started. The amino acid profile of diets A was based on

Table 1. Feed composition of diet A in growth phases 1 and 2 (0-21 and 22-43 days of age resp.).

	Phase 1 g/kg	Phase 2 g/kg
Wheat	248.7	246.1
Maize	216.4	174.6
Maize starch	-	98.5
Maizeglutenfeed	102.3	93.1
Lupins, white	85.1	24.7
Sunflower seed, extracted, decort.	80.0	18.2
Meat meal	70.0	70.0
Soya bean oil	75.0	73.8
Soya bean meal, solvent extracted	48.0	155.6
Maizeglutenmeal	38.5	36.2
Sugar	-	29.5
Minerals*	16.2	29.75
Vitamins*	5.0	5.0
Coccidiostat	0.5	0.5
Zinkbacitracin	0.3	0.3
Lysin-HCl	5.0	4.43
DL-methionine	2.83	2.09
Glycine	3.8	3.19
L-histidine	1.3	2.29
L-threonine	0.65	0.80
L-valine	0.51	1.35
Total	1000	1000

* Minerals and vitamins were added to comply with NRC standards.

the amino acid profiles of body accretion during 0-21 and 22-43 days of age respectively (ten Doeschate et al., 1991). Dietary composition during both growth phases is given in table 1. Table 2a shows analysed amino acid contents of diet A in both growth phases. In the three other test diets per growth phase the content of lysine or methionine was changed by exchanging lysine-HCL or DL-methionine with maize or maize starch. Diets B were calculated to contain 67% of the lysine content of diets A (all lysine-HCl was excluded). Diets C and D were calculated to contain 67% respectively 133% of the methionine content of diets A. Amino acid analysis of the test diets (Table 2b) confirmed the differences in diet composition.

Feed and water was availabale *ad libitum* except for two hours on the experimental day. At 12:30 on a pre-experimental day (according to the experimental design) birds were taken from the groups according to a randomized scheme. These chicks were placed pairwise in cages and fed the proper experimental diet. The feed was removed on the experimental day at 7:00 (start of a dark period). At 9:00, at the onset of a light period, the birds were allowed to consume the experimental diet for 30 minutes.

A growth trial (*exp. 1*) was performed along with the first breath test experiment (*exp. 2*). In *experiment 1* 12 groups of 10 birds were used to determine the effect of three test diets (B, C and D) on growth and feed conversion ratio from 0-21 and 22-43 days of age. Birds were fed diets B, C or D for the whole growth period. Bird weights and group feed intake were recorded weekly.

Breath test measurement

Approximately 1 μCi of [^{14}C] amino acid was injected subcutaneously in a fold of the skin just cranial to the right leg 60 minutes after the provision of a 30 minute meal. Determination of recovered $^{14}CO_2$ was according to the method of Schreurs et al (1992), modified for chicks as described earlier (ten Doeschate et al., 1995). To determine cumulative $^{14}CO_2$ recovery samples were regularly taken from the KOH-trap for 4.5 h after injection of label; each 30 minutes except for the first hour and last 30 minutes, when samples were taken each 15 minutes. At the end of the experiment birds were killed by providing a mixture of CO_2 and O_2 (70:30) to the respiration chambers. Excreta voided during $^{14}CO_2$ collection period were collected and [^{14}C]activity was counted as described earlier (ten Doeschate et al., 1995). Only small amounts (max. 3% of injected dose) of ^{14}C were found in the excreta, while treatment had no effect on this recovery. Therefore recovery of ^{14}C in excreta is not presented.

Table 2a. Amino acid content of diets A used in both growth phases of *exp. 1.* (g/100 g dm)

Amino acid:	1A 0-21 d	2A 22-43 d
Cys	0.37	0.33
Asx	1.87	1.80
Met	0.61	0.52
Thr	0.93	0.88
Ser	1.26	1.13
Glx	4.93	4.66
Gly	1.68	1.21
Ala	1.38	1.11
Val	1.22	1.15
Ile	0.90	0.84
Leu	2.11	1.90
Tyr	0.78	0.74
Phe	1.11	1.02
His	0.83	0.86
Lys	1.34	1.23
Arg	1.47	1.21
Pro	1.67	1.44

Experimental design

Exp. 2: The influence of dietary lysine or methionine content on $^{14}CO_2$ recovery from indicator amino acids was determined on three different days during 2 weeks in two growth phases. Starting from 12:30 on the pre-experimental day, birds on diet A were then fed diet A, B, C or D. [1-^{14}C]leucine or [1-^{14}C]valine were used as indicator amino acids. Measurements were carried out for all treatments (4 diets * 2 indicator amino acids) on each experimental day. This resulted in three replicates per age-period and treatment.

Exp. 3: Birds were fed diet B (low lysine) starting from 5, 3, 2 or 1 day before the breath test. $^{14}CO_2$ recovery from [1-^{14}C]leucine was determined on monday, wednesday and friday in four consecutive weeks (7-32 d of age). Two replicates for each treatment were done each experimental day.

Exp. 4: Birds fed on diet B (low lysine) for 5 days, were fed either diet A (normal lysine) or diet B on the pre-experimental day. On tuesday and thursday in four consecutive weeks (7-32 d of age) six $^{14}CO_2$ breath tests (three on diet A and three on diet B) were performed with [U-^{14}C]lysine. On these days, two additional breath tests were performed on chicks fed diet A using [1-^{14}C]leucine as indicator amino acid.

Table 2b. Methionine and lysine content of experimental diets used during growth phase 1 (0-21 d) and growth phase 2 (22-43 d) (g/100 g dm)

	amino acid Diet	methionine	lysine
	1A Normal	0.61	1.34
Phase 1	1B Low lys	0.61	0.94
0-21 d	1C Low met	0.39	1.38
	1D High met	0.82	1.35
	2A Normal	0.52	1.23
Phase 2	2B Low lys	0.52	0.85
22-43 d	2C Low met	0.32	1.18
	2D High met	0.67	1.21

Calculation and statistics

The data were analysed using Genstat (Genstat 5 committee, 1987). A cumulative recovery curve was calculated from the radioactivity of the samples. The recovery of $^{14}CO_2$ is defined as the mean of the last three values of the curve (4, 4.25 and 4.5 hours after injection). As described earlier (ten Doeschate et al., 1995) the recovery curve of each measurement could be fitted by a Gompertz equation (Genstat 5 committee, 1987). Parameters of the curves, estimated time

of maximal $^{14}CO_2$ recovery rate (t_{max}) and the time needed to reach half of total recovery (t_{half}), were analyzed.

Homogeneity of variances was checked by Bartlett's and Hartley's test (Seber, 1977; Digby et al.,1989). If one of these tests showed that variances were inhomogeneous between groups then analysis was performed using group variances given by Genstat to calculate SED for Student's t-test. Otherwise a normal ANOVA was performed.

Results

Exp. 1: growth and feed conversion ratio

Growth and feed conversion of the birds fed on the diets low in lysine (B) or low or high in methionine (C and D) is shown in table 3. A lower dietary lysine level (diet B) resulted in the lowest growth and highest feed conversion ratio. A low or high methionine content of the diet (diets C and D) did not influence growth or feed conversion ratio.

Breath test measurements

Experiment 2:

The analysis of results on a weekly basis showed that during the second growth phase (25-43 days of age), there was a significant ($P<0.05$) interaction between age-period (week) and diet for $^{14}CO_2$ recovery from [^{14}C]leucine. In figure 1 is shown, that there was a difference in $^{14}CO_2$ recovery between diet D and C at 25-29 days of age and not at 39-43 days of age. No other interactions were found. Further results were therefore combined over the two age-periods per growth phase. The effect of the dietary lysine or methionine level on recovery of $^{14}CO_2$ from [1-^{14}C]leucine and [1-^{14}C]valine (injected 60 minutes after the start of the meal) is given in table 4. On all diets, $^{14}CO_2$ recovery from [1-^{14}C]leucine was higher than from valine. During the first growth phase $^{14}CO_2$ recovery from [1-^{14}C]leucine as well as from [1-^{14}C]valine was highest ($P<0.001$) on diet B. Birds fed on diets C or D showed a response similar to birds fed on diet A. In the second

Table 3. Growth (grams per bird per day) and feed conversion ratio (feed/growth) for groups of birds receiving diets with low lysine (B), low methionine (C) and high methionine (D) (*Exp. 1*)

Diet:	Growth (n=4*10)				Feed conversion ratio (n=4)			
Age:	B	C	D	Sed	B	C	D	Sed
4-11	10.4	24.4	22.5	1.26 ***	2.09	1.44	1.51	0.116 ***
11-18	23.5	47.6	47.5	1.87 ***	1.94	1.55	1.43	0.070 ***
18-21	30.7	54.5	55.7	3.71 ***	1.87	1.68	1.37	0.086 NS
21-25	43.5	60.3	64.0	2.58 ***	1.89	1.95	1.86	0.036 NS
25-32	55.6	69.8	79.2	3.84 ***	2.04	2.05	1.80	0.065 **
32-39	64.2	73.3	74.3	3.80 NS	2.11	1.99	2.05	0.089 NS
39-43	78.4	80.3	90.7	3.26 **	2.07	2.11	2.07	0.09 NS
4-43	42.7	58.3	62.0	1.79 ***	2.03	1.88	1.80	0.038 ***

Significance levels of diet are given for each age-period. (NS=P>0.05; *:P<0.05; **:P<0.01; ***:P<0.001)

growth phase no significant effects of diet on $^{14}CO_2$ recovery from [1-^{14}C]leucine or [1-^{14}C]valine were noted.

For [1-^{14}C]valine t_{max} and t_{half} were influenced by age-period within a growth phase ($P<0.05$). In table 5, data for both periods per growth phase are presented. During both growth phases t_{max} and t_{half} increased when birds became older, which means that release of $^{14}CO_2$ from [1-^{14}C]valine was decelerated. This effect was not found for [1-^{14}C]leucine. The parameters describing the shape of the fitted curves were not influenced by diet and the effect of diet on t_{max} and t_{half} is therefore not shown.

Table 4. Recovery of $^{14}CO_2$ from [1-^{14}C]leu or [1-^{14}C]val after a meal of a normal diet (A) or a diet with low lys content (B, 67 % of A), low met content (C, 67 % of A) or high met content (D, 133 % of A) during two growth phases (5-22 and 25-43 days of age). (Mean; n = 8; val, diet B/C 25-43 days of age n = 7) (*Exp. 2*)

		Diet				
	Age (d)	A	B	C	D	Sed
Leu	5-22	18.1^a	23.4^b	18.3^a	16.6^a	1.44
	25-43	15.1	16.7	15.9	14.0	1.09
Val	5-22	11.2^a	17.2^b	12.0^a	11.2^a	1.10
	25-43	11.2	12.1	12.4	10.4	1.17

Means with different superscripts in a line were significantly ($P<0.05$) different.

Table 5. Parameters of curves fitted according to a Gompertz equation for the recovery of $^{14}CO_2$ from [1-^{14}C]valine. Diets A,B,C and D combined. (Mean; n = 16; 39-43 days of age: n = 14) (*Exp. 2*)

Age (days)	5-8	19-22	25-29	39-43
Time (h) of maximal $^{14}CO_2$ recovery rate	0.85	0.95	1.01	1.09
Sed + sign.		0.0345 **		0.0341 *
Time to reach half value (h)	1.06	1.17	1.24	1.33
Sed + sign.		0.0392 *		0.0424 NS

Comparisons were made within growthphase (5-22 and 25-43 days of age). (NS:$P>0.05$; *:$P<0.05$; **:$P<0.01$)

Experiment 3:

The effect of length of adaptation period on $^{14}CO_2$ recovery was tested with [1-^{14}C]leucine. The results are given in table 6. The length of the adaptation period had no clear effect on recovery of $^{14}CO_2$. Irrespective of the length of the adaptation period lower recoveries were found when birds, within both growth phases, became older.

Table 6. Recovery of $^{14}CO_2$ (%) from [1-^{14}C]leucine when a diet with low lysine content (B) is fed for 1, 2, 3 or 5 days before breath test measurement. (Mean; n = 6) (*Exp. 3*)

Period Age (d)	1 day	2 days	3 days	5 days	Sed
7-11	22.7	22.3	24.9	22.5	1.57
14-18	20.2	20.2	22.2	22.2	1.64
21-25	18.1	17.7	19.5	18.8	1.20
28-32	15.5	15.4	16.2	16.0	1.50
7-18	21.5	21.3	23.5	22.3	1.11
21-32	16.8	16.5	17.8	17.4	0.93

Student's t-test (double-sided) performed within age-period (df = 5) or growth phase (df = 11) detected no significant ($P < 0.05$) effects.

Table 7. Recovery of $^{14}CO_2$ from [1-^{14}C]leucine when birds adapted for 5 days to a low lysine diet (B) were fed a normal (A, *exp. 4*) or low (B, *exp. 3*) lysine diet during a meal and 20 hr preceeding breath test.

Diet Age (d)	A, normal lysine (n = 4)	B, low lysine (n = 6)	Sed	sign. level
7-11	20.4	22.5	1.32	$P > 0.10$
14-18	17.4	22.2	0.60	$P < 0.01$
21-25	17.0	18.8	0.94	$P < 0.10$
28-32	14.0	16.0	1.29	$P < 0.10$
7-18	18.9	22.3	0.89	$P < 0.01$
21-32	15.5	17.4	1.01	$P < 0.10$

Student's t-test (one-sided) was performed within age-period (df = 3) or growth phase (df = 7).

Experiment 4:

$^{14}CO_2$ recovery from [1-^{14}C]leucine in birds refed diet A (*exp. 4*) or B (*exp. 3*) after 5 d adaptation to diet B is shown in table 7. Although measurements were made on separate days, the difference between diets is thought to be due to

dietary lysine content, because no effect of measurement day has been detected in the other experiments. From table 7 it is clear that in birds adapted for 5 days to a low-lysine diet $^{14}CO_2$ recovery from [1-^{14}C]leucine was lower if a normal lysine diet was fed during the pre-experimental day.

Table 8. Recovery of $^{14}CO_2$ from [U-^{14}C]lysine when birds adapted for 5 days to a low lysine diet (B) were fed a normal (A) or low (B) lysine diet during the last 20 hr preceeding breath test. (Mean; n = 6) (*Exp. 4*)

Diet / Age (d)	A, normal lysine	B, low lysine	Sed	sign. level
7-11	1.27	0.72	0.18	$P < 0.05$
14-18	2.75	0.77	0.27	$P < 0.001$
21-25	3.83	1.02	0.30	$P < 0.001$
28-32	3.38	1.25	0.18	$P < 0.001$
7-18	2.01	0.74	0.27	$P < 0.001$
21-32	3.61	1.13	0.19	$P < 0.001$

Student's t-test (one-sided) was performed within age-period (df = 5) or growth phase (df = 11).

Table 8 shows the comparison for $^{14}CO_2$ recovery from [U-^{14}C]lysine, when birds were fed a normal (A) or a low lysine diet (B). In figure 2 the influence of age on $^{14}CO_2$ recovery from [U-^{14}C]lysine in both dietary groups is shown. The $^{14}CO_2$ recovery was influenced by age of the birds when fed diet A. Only for 8 d old chicks the $^{14}CO_2$ recovery was independent of dietary lysine content. Older birds did react to a higher dietary lysine level by a higher $^{14}CO_2$ recovery.

Discussion

Indicator method

The present study showed, that lowering the content of an essential amino acid in the diet can result in a higher $^{14}CO_2$ recovery from an injected [^{14}C] labeled indicator amino acid. For dietary lysine level this was only found during the first growth phase (table 4: 5-22 d). This means that during this growth period $^{14}CO_2$

recovery from indicator amino acids can be used to study requirement of the limiting amino acid. Over the growing period as a whole, differences in dietary methionine level were not reflected in differences in $^{14}CO_2$ recovery from [1-^{14}C]leucine or [1-^{14}C]valine. Growth and feed conversion ratio were correspondingly also not affected. This suggests, that even the low methionine content of diet C was sufficient for most of the time during both growth phases. However, in chicks of 25-29 days of age a significant lower $^{14}CO_2$ recovery from [1-^{14}C]leucine was found when dietary methionine level was higher (Fig. 1). Thus, at that age, at the lower methionine levels, dietary supply was not sufficient to reach maximal protein synthesis. This temporary high methionine requirement may be related to a relative high feather growth in this period, increasing the requirement of sulphur-containing amino acids. Because measurement of $^{14}CO_2$ recovery is a short term measurement this temporary high requirement is not necessarily observed in growth or feed conversion ratio when measured in a longer term growth study.

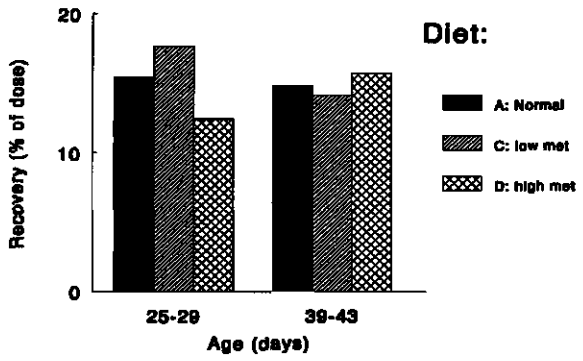

Figure 1. Interaction between age (25-32 and 39-43 d of age) and dietary methionine level on recovery of $^{14}CO_2$ from [1-^{14}C]leucine. (*Exp. 2*)

In *experiment 4* the $^{14}CO_2$ recovery from [1-^{14}C]leucine on diet A (normal lysine) or diet B (low lysine) was determined in birds which were fed on diet B (low lysine) for 5 days prior to the test diets. A comparison between *table 4* and *table 7* shows, that the diet fed prior to the test diet did not have any influence on the recovery data. In both experiments, the effect of lysine content of the meal on the $^{14}CO_2$ recovery was similar. Therefore $^{14}CO_2$ recovery after a meal is primarily related to the short term amino acid supply and rather independent of the longer term nutritional status. Similar results were found for rats by Weijs (1993).

The experiments showed, that $^{14}CO_2$ recovery from an indicator amino acid changes when the level of a dietary limiting amino acid is altered. The coefficient of variation was in this study about 10-15 %, therefore the sensitivity is not very high. The lysine content of diet B is two thirds of that of diet A; $^{14}CO_2$ recovery from [1-^{14}C]leucine on diet B varied between 110 and 130 % of diet A. Smaller

differences in dietary lysine level will result in even smaller differences in $^{14}CO_2$ recovery and thus dietary effects may be hard to detect. The $^{14}CO_2$ breath test measurement determines which part of the labeled amino acid is recovered as $^{14}CO_2$. A lower $^{14}CO_2$ recovery suggests a higher utilization for protein synthesis of the tested amino acid. The $^{14}CO_2$ recovery is a measure of the percentage oxidation of the labeled flux. Labeled flux consists of endogenous amino acid flux and of dietary amino acid supply. Thus not necessarily $^{14}CO_2$ recovery should rise to the same extent as the relative oversupply of leucine or valine. In addition, oxidation of various amino acids may show differences in sensitivity for metabolic perturbation due to their dietary oversupply (Schreurs et al., 1994). Moreover, utilization and oxidation may also vary over the day as a result of diurnal cycling. In our broiler chicks (1L:2D), the rather constant dietary supply of amino acids is not expected to cause significant diurnal cycling and therefore a rather constant level of amino acid oxidation might be expected. This was confirmed in a previous paper (ten Doeschate et al., 1995) where it was shown that, based on $^{14}CO_2$ recovery after different times of injection relative to the meal, in chicks oxidation shows minor fluctuations during the day.

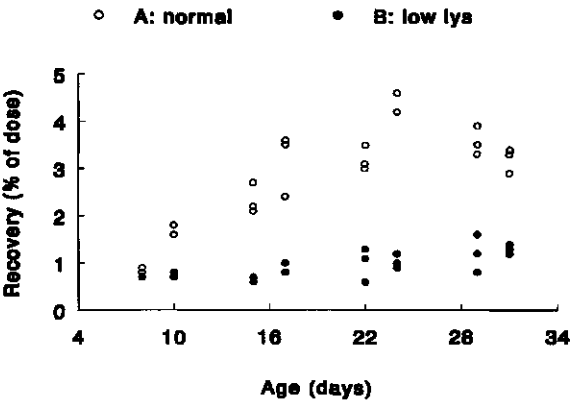

Figure 2. Effect of age on recovery of $^{14}CO_2$ from [U-^{14}C]lysine injected after a meal of a diet with a normal (A) or low (B) lysine content. (*Exp. 4*)

The difference in $^{14}CO_2$ recovery from leucine and valine can be explained by differences in relative oversupply of both amino acids. Although the feed was composed to supply all amino acids in a pattern reflecting body accretion, the ratio between leucine and valine in the diet did not exactly parallel the ratio in body accretion. During the first growth phase (0-21 d) the ratio leucine:valine in body accretion is 1.25 (ten Doeschate et al., 1991) whereas this ratio in the diet was 1.73 (table 2a). In the second growth phase (22-43 d) these ratios where 1.24 and 1.65 respectively. Thus the relative oversupply of leucine was larger than that of valine. In order to maintain constant amino acid levels in the free amino acid pool, oxidation of leucine should thus be higher than oxidation of valine. In the second

growth phase, relative leucine oversupply was less than in the first phase therefore a smaller difference in $^{14}CO_2$ recovery between leucine and valine, as observed, can be expected.

The increase with age of the parameters (t_{max}, t_{half}) describing the shape of the recovery curve from [1-^{14}C]valine, as shown in table 5, is not readily explainable. For [1-^{14}C]leucine, these parameters, measured under similar conditions in the same experiment, did not show this effect. This rules out an artefact caused by some factor not related to [1-^{14}C]valine. From these data it is concluded, that in older chicks oxidation of valine occurs at a slower rate than in younger chicks. An explanation might be a substantial change in flux size or a reduced sensitivity of the catabolic enzymes for metabolic perturbation.

Auto-indicator method

A $^{14}CO_2$ recovery of 2-4 % of [U-^{14}C]lysine was found , when a diet adequate in lysine was fed, while a diet with a low lysine content resulted, as expected, in a lower recovery (less than 1 %). Similar results were extracted from a previous experiment (ten Doeschate et al., 1995).

The influence of age on $^{14}CO_2$ recovery from [U-^{14}C]lysine, as shown in *fig 2*, probably reflects the changes in lysine requirement during growth. During the first growth phase (0-21 d) birds on the high lysine diet showed a slight rise with age in $^{14}CO_2$ recovery from [U-^{14}C]lysine. After 21 d of age, when the dietary lysine level was lowered in both diets, $^{14}CO_2$ recovery remained essentially constant. This suggests, that up to three weeks of age lysine requirement is lowered gradually while after three weeks of age the dietary lysine level may be held constant. It is concluded that the auto-indicator method can be used to determine adequacy of dietary amino acid level.

Adaptation to diet

No effect of length of adaptation to diet B on $^{14}CO_2$ recovery from [1-^{14}C]leucine was found in the range of 1 to 5 d of adaptation. Similarly, the use of indicator amino acids or the auto-indicator method in birds adapted to either a low or a normal dietary lysine level revealed no effect of adaptation to the diet. Chicks reacted similarly on a meal (plus a pre-experimental feeding period of 20 h) of a high or low lysine diet irrespective the diet fed during the adaptation period. This suggests that chicks either adapt very fast (within one day) or that enzyme

systems are not primarily set by lysine content of the diet but more by acute supply. In addition it should be realized that in our experiments digestive enzymes did not have to adapt to changes in dietary composition because, apart from free amino acids, this was constant. When an extreme low lysine diet was fed to chicks Akinwande & Bragg (1985) reported a lower liver protein turnover, measured from the half live of [U-^{14}C]lysine. This suggests that lysine-deficient chicks have the capacity to retain lysine in body tissues. Picard et al. (1993) showed that broiler chicks reacted to amino acid deficiencies within a short period (hours). We therefore tend to conclude that utilization of amino acids adapts very quickly to acute amino acid supply.

Conclusions

At a normal dietary lysine level $^{14}CO_2$ recovery from both [1-^{14}C]leucine and [1-^{14}C]valine was reduced compared to a low dietary lysine level. This indicates a higher utilization of both amino acids for protein synthesis on the normal lysine diet. The sensitivity to measure dietary adequacy of amino acid supply is however limited. The age-dependant effect of dietary methionine level indicates that the method is especially suited for assessment of short term effects in amino acid utilization. The present study shows that for the determination of short term effects of dietary amino acid levels indicator as well as auto-indicator amino acids can be used.

Adaptation of the bird to dietary lysine levels is either very quick (within a day) or does not influence $^{14}CO_2$ recovery from [^{14}C]amino acids. The $^{14}CO_2$ recovery from [^{14}C]amino acids is essentially determined by acute amino acid supply e.g. the dietary amino acid level fed during the meal and a 20 h period preceeding the $^{14}CO_2$ breath test measurement.

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Chapter 5

NITROGEN EFFICIENCY IN TWO GENOTYPES OF BROILER CHICKS FED DIETS DIFFERING IN PROTEIN:ENERGY RATIO AND AMINO ACID SUPPLEMENTATION.

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Summary

Nitrogen efficiency (deposition as a percentage of intake) of broiler production can be improved either by a genetic change in efficiency or by imroved dietary composition. An improved dietary amino acid pattern can be based on the amino acid pattern of body accretion. To improve nitrogen efficiency or growth performance it may be necessary to supplement this body accretion based pattern with specific amino acids (Methionine or Arginine) or with non-essential amino acids. The level of protein/energy (P/E) ratio will affect N-efficiency. Furthermore, there may be an interaction between dietary composition and genotype.

Growth performance and nitrogen efficiency were studied in both sexes of two genetic different broiler lines, fed 12 diets differing in amino acid composition and P/E ratio. Three diets, of which the amino acid pattern was similar to that of body accretion, differed only in P/E ratio (High: 0-21 days of age and 22-slaughter age: 17.8 and 19.3 g CP/MJ AME respectively; Medium and Low: 94 % and 87 % of High). Six other diets, based on the medium P/E ratio, were supplemented with either Met, Arg, Glu, Gly, Glu + Gly or Met + Arg + Glu + Gly. Two diets were based on the low or high P/E ratio and supplemented with all four amino acids. Diet 12 was a control diet with a semi-practical composition. Male chicks of a commercial broiler hybrid (Com) were fed all diets. Male chicks from a population selected for low feed conversion ratio (FC) were fed the three diets differing in P/E ratio plus the medium P/E diet supplemented with all four amino acids. This latter diet and the unsupplemented medium P/E diet were fed to female chicks of both genotypes.

N-efficiency was not improved by amino acid supplementation. Met-supplementation improved growth rate at similar N-efficiency while other supplementations negatively affected N-efficiency. Both genotypes reacted similar to differences in dietary composition. This supports the hypothesis that the amino acid pattern of body accretion is a good reference for dietary amino acid pattern. Female chicks also reacted, at a lower N-efficiency level, similar to differences in dietary composition as male chicks. Growth performance was lower at low P/E ratio while N-efficiency was lower at high P/E ratio. Maximal growth performance and maximal N-efficiency can thus not be attained simultaneously. It is calculated that, compared to male Com chicks fed the control diet, feeding the diet with highest N-efficiency (low P/E ratio) will result in a reduction of N-excretion of 11 %. When this diet is fed to male FC chicks, N-excretion will be only 67 % of that of male Com chicks fed the control diet. It is concluded that both dietary and genetic improvement will result in lower N-excretion.

Introduction

Animal production can be considered as a way to convert relatively low value vegetable protein such as residues from human food production into more appreciated animal protein. In this conversion a considerable part of the nitrogen is lost (Coppoolse et al., 1990). The process of nitrogen conversion can be split into two processes, firstly the digestion of the protein (amino acid availability) and secondly utilisation of absorbed amino acids. Chicks excrete the absorbed nitrogen not used for body protein accretion as uric acid. After excretion microbial action can easily convert uric acid to ammonia which subsequently leads to ammonia emission to the air. Excess nitrogen excretion and ammonia emission causes an environmental problem. A logical attempt to reduce this problem is to improve the efficiency with which dietary nitrogen is deposited into animal protein. In this way nitrogen excretion per kilogram animal protein produced will be lowered. Some losses might be inevitable while others can be reduced by feeding a diet with an amino acid pattern well balanced relative to the birds' requirements. In the past, requirements for amino acids have been largely considered as minimum requirements. However, since safety margins tend to be used in feed formulation, this approach leads inevitably to oversupply. When formulating feeds one should make sure that not only minimum requirements are met but also that no relative surpluses of amino acids are fed. For broiler chicks determination of the amino acid profile of body accretion (ten Doeschate et al., 1995) can be used as a basis for amino acid requirements. Because of the high growth rate of broiler chicks, total requirements are mainly determined by requirements for protein accretion. For some amino acids this might be less valid than for others, which may imply that for some amino acids maintenance requirements do affect total requirements. This means that providing more of those amino acids in the diet than the amino acid pattern of body accretion indicates may have a positive effect on nitrogen efficiency. Methionine (as methyldonor) and arginine (which, due to lack of carbamoyl phosphate synthase, can not be formed in birds) may be amino acids which would exhibit such an effect.

Furthermore, Moran et al. (1992) suggested that lowering the protein level may result in a deficiency of non-essential amino acid nitrogen. This deficiency might be compensated by adding a mixture of glutamic acid (Glu) and glycine (Gly) to the diet. In this mixture glutamic acid should cover for Glx, Asp, Ala and Pro while glycine should cover for Gly and Ser.

Performance of broiler chicks is affected by dietary protein/energy (P/E) ratio as shown by several previous reports (Rosebrough et al., 1987; Summers et al., 1988; Bartov, 1989; Huyghebaert et al., 1991; Bartov, 1992; Roth et al., 1993). However, often P/E ratio is varied by a change in ingredients which simultaneously results in a change in dietary amino acid pattern. Furthermore, performance is mostly monitored as growth rate or feed efficiency and without considering nitrogen efficiency.

The three subjects mentioned above (use of amino acid pattern of body accretion as basis for diet formulation, deficiency of non-essential amino acid nitrogen and P/E ratio) were studied in a series of trials. To investigate the use of carcass amino acid pattern as basis for diet formulation we used chicks from two genetic stocks, one commercial and one selected for low feed conversion ratio (FCR).). The birds selected for low FCR (FC birds, from the selection experiment of Leenstra (Leenstra & Pit, 1981)) have a lower intrinsic growth rate which would, as a result of a longer period required to attain the desired slaughter weight, lead to higher maintenance requirement compared to commercial broilers. To test the possible deficiency of non-essential amino acids, the efficiency of nitrogen utilisation was determined in birds fed diets based on the amino acid pattern of body accretion with or without extra supplementation of Glu and Gly.

The effect on nitrogen efficiency of three P/E ratio's was studied for three diets attained by dilution of a basal diet with a nitrogen free iso-energetic mixture. The P/E ratio might, due to changes in allometric growth of body components (Haakansson et al., 1978), affect whole body amino acid composition. In that case, the amino acid pattern of body accretion used for diet formulation would no longer be valid. For this reason, in three week old chicks fed the three P/E ratio's the whole body amino acid pattern was checked.

Materials and methods

Birds and husbandry

Male and female chicks of two genotypes were used. Most of the birds were from a commercial broiler hybrid (Euribrid, Boxmeer). Birds selected for efficient food conversion between 21 and 42 days of age were used in some parts of the experiment. These birds were taken from the 12th generation of the selection experiment described by Leenstra & Pit (1987). The first line is designated by Com

whereas the second line is designated by FC. Day-old chicks were sexed, wing-banded and vaccinated against infectious bronchitis and Newcastle disease. At 17 days of age, birds were inoculated against bursal (Gumboro) disease. Birds were housed in 120 litter floor pens (75*97 cm) in an environmentally controlled broiler house. Pens were arranged in six rows. Treatments were randomized within rows to attain a randomized block design with row as factor block. Per pen, 15 Com or 12 FC birds were placed. Environmental temperature decreased gradually from 32 °C for day-old chicks to 18 °C at 6 weeks of age. After 3 d of continuous light, birds were kept under a lighting regime of 1L:2D to prevent leg disorders and to stimulate regular feed intake. Birds had continuous access to feed and water.

Diets

Based on critical changes in whole body amino acid pattern the growth period was divided into two growth phases: 0-21 days of age and 22 days of age until slaughter (ten Doeschate et al., 1991) designated phases 1 and 2 respectively. The amino acid pattern of the basal diet was based on the amino acid pattern of body accretion in the respective periods (ten Doeschate et al., 1995). Three P/E ratio's were attained by dilution of the basal diet in two steps with an iso-energetic N free dilution mixture (88.6 % corn starch, 8.8 % cellulose + minerals, vitamins and additives in a concentration equal to the basal diet.) This resulted in diets 1 (High P/E ratio = 100%), 2 (Medium P/E ratio = 94%) and 3 (Low P/E ratio = 87%). High P/E ratio contained 17.8 and 19.3 grams crude protein per MJ Apparently Metabolisable Energy (AME) in both growth phases, respectively.

To test whether addition of individual or a combination of amino acids would exert a positive influence on production parameters or nitrogen efficiency several variations on diet 2 were composed. The amino acids supplemented were exchanged against the dilution mixture. The effect of essential amino acid supplementation to cover maintenance requirements was tested for methionine in diet 4 (0.10 g/100 g diet) and for arginine in diet 5 (0.1012 and 0.0932 g/100 g diet in phases 1 and 2, respectively).

The effect of supplementation with non-essential amino acid nitrogen was studied in diets 6, 7 and 8. Diet 6 contained extra glutamic acid (0.5278 and 0.4698 g/100 g diet in phase 1 and 2, respectively); diet 7 extra glycine (0.1951 and 0.1636 g/100 g diet); diet 8 extra glutamic acid and glycine in the same amounts as mentioned before.

Table 1. Nutrient contents (g/kg) of two selected diets. Apparent ileal digestible amino acid contents calculated from amino acid contents of the foodstuffs combined with digestibility coefficients determined according to ten Doeschate et al. (1993).

	Phase 1	(0-21 d)	Phase 2	(22-43 d)
AA Diet:	2	9	2	9
Cys	2.39	2.39	2.21	2.21
Asp	13.43	13.43	11.5	11.5
Met	4.07	5.06	5.22	6.21
Thr	6.09	6.09	6.22	6.22
Ser	6.53	6.53	5.77	5.77
Glu	25.27	30.54	22.41	27.11
Pro	10.87	10.87	9.84	9.84
Gly	13.05	15.00	10.08	11.71
Ala	8.65	8.65	8.39	8.39
Val	9.25	9.25	9.80	9.80
Ile	6.66	6.66	7.13	7.13
Leu	12.27	12.27	11.55	11.55
Tyr	5.55	5.55	5.05	5.05
Phe	7.04	7.04	6.27	6.27
His	5.70	5.70	6.15	6.15
Lys	11.25	11.25	10.53	10.53
Arg	11.15	12.16	10.40	11.33
Trp	1.97	1.97	2.09	2.09
AME (MJ)	11.95	11.95	12.1	12.1
CP (N*6.25)	216	224	204	209

The combined effect of both essential and non-essential amino acid supplementation (full amino acid supplementation) was tested at each P/E ratio. Diet 9 was based on diet 2, supplemented with all four amino acids. Diet 10 was based on diet 1 which was supplemented with the same amount of amino acids as was diet 9. Diet 11 was composed similarly with diet 3 as basis. The levels of supplementation of Glu and Gly were chosen in order to raise the non-essential amino acid content of the medium P/E diet up to that of the high P/E diet. Diet 12

was a semi-practical control diet with an AME of 13.2 MJ and 220 g/kg crude protein. Composition and digestibility of this diet were described previously (ten Doeschate et al., 1993). Ileal amino acid digestibility coefficients of the natural feedstuffs used in diets 1 to 11 were determined in a separate experiment according to the method described by ten Doeschate et al. (1993). From these the levels of ileal digestible amino acids in the experimental diets were calculated. It was assumed that synthetic amino acids are for 100 % digestible. Table 1 shows nutrient (ileal digestible amino acids, CP and AME) content of diet 2 (Medium P/E) and diet 9 (Medium P/E plus amino acids) in both growth phases. The latter was chosen because it shows the levels of the four added amino acids to diet 2.

Measurements

Bird weights and feed consumption were measured weekly. At 21 and 43 days of age per pen three birds were taken for carcass analysis. At these days birds were weighed per cage before and after removal of birds. Before weighing, feed was withdrawn for 5 hours. Birds for analysis were sacrificed by suffocation in CO_2. Carcasses were stored at -18 °C until further sample preparation. After 43 days of age the experiment was terminated for four treatment groups (male com birds fed diet 1, 4, 9 or 10); the remaining groups were allowed to grow until the mean bird weight was approximately equal to that of the first four finished groups ($\pm$ 2500 g). At the final day, birds were weighed and again three birds were taken for carcass analysis.

Sample preparation and analysis

Carcass samples (3 birds/sample) were prepared for analysis according to a modification of the method of Scheele and Jansen (1971). Carcasses were minced in a meat cutter while still frozen. A subsample was mixed with celite to bind and disperse fat and then freeze-dried. After freeze-drying, all samples were ground to pass a 1.5 mm sieve.

Nitrogen content was determined with a standard Kjehldahl method. Analytical results as determined in the samples were corrected according to the relative amount of celite added to the minced chicken. Amino acid contents were determined in male Com birds fed diets 1, 2 and 3 at three weeks of age. For amino acid analysis an acid and separate oxidative hydrolysis was performed. After

Table 2. Experimental design: For each combination of diet fed and genotype/sex it is shown in which statistical model the results of these combination are analyzed. Combinations where no model number is given were not present in the experiments.

Diet	Genotype (sex) Com (male)	Com (female)	FC (male)	FC (female)
1) P/E = 100	[1], [3], [5]		[1]	
2) P/E = 92	[1],[2],[3],[4],[5]	[2]	[1],[2]	[2]
3) P/E = 85	[1],[3],[5]		[1]	
4) 2 + Met	[4]			
5) 2 + Arg	[4]			
6) 2 + Glu	[4]			
7) 2 + Gly	[4]			
8) 2 + Glu + Gly	[4]			
9) 2 + Met + Arg + Glu + Gly	[2],[3],[4]	[2]	[2]	[2]
10) 1 + Met + Arg + Glu + Gly	[3]			
11) 3 + Met + Arg + Glu + Gly	[3]			
12) control	[5]			

Models: (For explanation: *Experimental design*)

[1] $Y = \mu + Block + P/E + G + P/E * G + e$

[2] $Y = \mu + Block + G+S + AA + G*S + G*AA + S*AA + G*S*AA + e$

[3] $Y = \mu + Block + P/E + AA + P/E*AA + e$

[4] $Y = \mu + Block + AAsup + e$

[5] $Y = \mu + Block + Diet + e$

passage through a LC-ion column (Beckman LC 5001) amino acids were derivatised with ninhydrin . Norleucine was used as internal standard. The estimated contents of Thr, Ser, Ile and Val were corrected for incomplete recovery after hydrolysis according to Slump (1969) and thus multiplied by the following factors: 1.05, 1.10, 1.08 and 1.07, respectively. Amino acid contents in the birds were expressed on a weight basis (g/100 g) relative to total amino acids recovered.

Experimental design and statistics

The parameters were calculated for the first growth phase (0-21 days of age), the second growth phase (22-43 days of age) and for the entire growth period until equal weight (2500 g). These periods are designated as GP1, GP2 and TGP, respectively. Feed conversion ratio was calculated based on the mean daily feed intake per bird per pen combined with mean daily gain. Nitrogen efficiency was calculated from ileal digestible nitrogen intake combined with nitrogen accretion based on analyzed nitrogen content in the carcass samples. Data were analyzed using Genstat (Genstat 5 committee, 1987, 1993) as a statistical program. In general, ANOVA was performed using a randomized block design, followed by a paired t-test if F-test indicated significant differences.

In total there were 20 treatment groups, 12 Com male, 2 Com female, 4 FC male and 2 FC female. Female chicks were fed diets 2 and 9 (Medium P/E ratio without and with amino acid supplementation). Male FC chicks were fed diets 1, 2, 3 and 9 (All P/E ratio's plus the medium P/E ratio AA-supplemented. Male Com birds were fed all 12 diets. The experimental designs and the statistical models in which the treatment groups were used are displayed in table 2.

The entire experiment can be divided into several sub-experiments which were analyzed with five statistical models. According to the effects and interactions of interest a particular model was used. The five models used are described below.

1) Effect of P/E ratio and genotype in male chicks

Diets 1, 2 and 3 were fed to male birds of Com or FC genotype. The statistical model used for ANOVA was:

$$Y = \mu + \text{Block} + P/E + G + P/E*G + e$$

[Model 1]

where Y = measured response; μ = grand mean; Block = one of 6 rows of pens in poultry house; P/E = protein/energy ratio at three levels; G = genotype: FC or Com; P/E * G = interaction between P/E and genotype; e = random error.

2) Effect of genotype, sex, and full amino acid supplementation.
Diet 2 or 9 were fed to male and female chicks of both Com as FC genotype. The following model was applied:

$$Y = \mu + Block + G + S + AA + G*S + G*AA + S*AA + G*S*AA + e$$
[Model 2]

where for denominators not previously used holds that: S = sex: male or female; AA = amino acid supplementation; G*S = interaction between genotype and sex.

3) Effect of P/E ratio and full amino acid supplementation.
Diets 1, 2, 3, 10, 9 and 11 were fed to male chicks of Com genotype. The model used was:

$$Y = \mu + Block + P/E + AA + P/E*AA + e$$
[Model 3]

4) Effect of supplementation with various amino acids.
The effect of supplementation with various amino acids, apart and as a mixture was tested in male Com chicks with the medium P/E ratio as basal diet. Diets used were: 2, 4, 5, 6, 7, 8 and 9. This leads to the following model:

$$Y = \mu + Block + AAsup + e$$
[Model 4]

where AAsup is a factor with 7 levels; one for each dietary amino acid supplementation.

5) Comparison of control diet with experimental diets 1, 2 and 3.
The control diet which was previously tested (ten Doeschate et al., 1995) was compared to the three P/E ratio's in which the AA-pattern was based on the AA-pattern of body accretion. Only male Com chicks were used for this comparison.

$$Y = \mu + Block + diet + e$$
[Model 5]

where diet is a factor with four levels.

6) Amino acid analyses were performed in three week old male Com birds fed diets 1, 2 and 3 (High, medium and low P/E). The effect of dietary treatment was determined to see whether the amino acid pattern was affected by P/E ratio. The model used was:

$Y = \mu + \text{Block} + P/E + e$
[Model 6]

Results

Growth

Growth rate (g/day) of the birds during both growth phases and over the entire growth period is given in table 3. The statistical evaluation was done according to the five models described above. According to the first model it is shown that in GP1 a lower P/E ratio resulted in a decreased growth rate for Com as well as FC birds. In GP2 a decreased growth rate was only found for FC birds which resulted in a significant interaction between genotype and P/E ratio. For TGP (until similar weight) this interaction was not significant ($P=0.052$). The overall effect of genotype and P/E ratio was that a lower P/E ratio as well as FC birds resulted in a lower growth performance.

According to the second model, genotype as well as sex affected growth performance. In general, males grew faster than females but in FC birds this difference was smaller (in GP1 even not significant) than in Com birds which explains the observed interaction between genotype and sex. In GP2 interaction between genotype and AA-supplementation indicated that in FC birds AA-supplementation had more effect than in Com birds. Amino acid supplementation (Met, Arg, Glu and Gly combined) increased growth rate (mean over genotypes and sexes) from 25.1, 63.7, 49.4 to 27.9, 65.6, 50.4 during the three respective growth periods.

Analysis according to the third model revealed that only during GP1 there was a significant positive effect of AA-supplementation and a higher P/E ratio. In older birds or when TGP was analyzed this effect was no longer detectable.

Supplementation with individual amino acids or combinations of amino acids did have a significant effect in GP1 but not in older birds. Diet 4 and 9, which were both supplemented with methionine, resulted in a better growth performance

Table 3. Growth rate of birds of both sexes of two genotypes fed diets differing in amino acid pattern or P/E ratio. F-probabilities are shown according to five models of analysis. The column *age* shows at which age birds weighed ± 2500 g (final weight).

Genotype	Sex	Die	Growth rate (g/d) during: GP1 (0-21 d)	GP2 (22-43 d)	TGP (0d - 2500	Age (d)
Com	male	1	35.7$^{d,)],=}$	77.6$^{c,),-}$	57.1$^{c,),-}$	43
		2	34.1$^{d,v,u,),\#,@,+,=}$	78.9$^{c,w,),\#,-}$	58.1$^{c,w,),\#,-}$	45
		3	31.1$^{c,),-}$	78.0$^{c,),-}$	55.7$^{c,),-}$	45
		4	36.0$^{@}$	79.8$^{\#}$	58.4$^{\#}$	43
		5	33.3$^{\#}$	80.0$^{\#}$	58.4$^{\#}$	45
		6	33.7$^{\#}$	78.0$^{\#}$	57.2$^{\#}$	45
		7	33.4$^{\#}$	77.8$^{\#}$	56.9$^{\#}$	45
		8	33.0$^{\#}$	80.2$^{\#}$	57.8$^{\#}$	45
		9	36.0$^{u,)],@}$	79.3$^{w,),\#}$	58.2$^{w,),\#}$	43
		10	37.5$^{]}$	78.3$^{]}$	58.3$^{]}$	43
		11	34.9$^{)}$	78.3$^{)}$	58.0$^{)}$	45
		12	32.3$^{-,+}$	73.7^{-}	54.7^{-}	46
FC	male	1	22.2^{b}	64.3^{b}	49.4^{b}	51
		2	19.3^{a,zy}	59.8^{b,y}	47.5^{b,y}	53
		3	18.1^{a}	52.0^{a}	44.9^{a}	55
		9	22.1^{x}	62.9^{yx}	48.9^{yx}	51
Com	femal	2	29.1^{w}	65.2^{x}	50.2^{yx}	50
		9	32.5^{v}	66.0^{x}	51.8^{x}	49
FC	femal	2	17.8^{z}	51.1^{z}	41.9^{z}	56
		9	21.0^{yx}	54.3^{z}	42.9^{z}	56

Model	Facto	F-probabilities/sed		
1	P/E	<.001 / .412	<.001 / .979	<.001 / .668
	Genotype	<.001 / .337	<.001 / .799	<.001 / .545
	P/E * Genotype	0.086 / .583	<.001 / 1.385	0.052 / .945
2	AA-sup.	<.001 / .301	0.001 / .548	0.008 / .361
	Genotype	<.001 / .301	<.001 / .548	<.001 / .361
	Sex	<.001 / .301	<.001 / .548	<.001 / .361
	AA-sup. * Geno	0.563 / .426	0.026 / .775	0.633 / .511
	AA-sup. * Sex	0.160 / .426	0.765 / .775	0.436 / .511
	Genotype * Sex	<.001 / .426	<.001 / .775	0.079 / .511
	A * G * S	0.348 / .602	0.925 / 1.097	0.208 / .722
3	AA-sup.	<.001 / .339	0.680 / 1.069	0.068 / .625
	P/E ratio	<.001 / .416	0.653 / 1.310	0.266 / .765
	A * P	0.040 / .588	0.990 / 1.852	0.370 / 1.082
4	AA-sup.	<.001 / .534	0.639 / 1.620	0.626 / .971
5	Diet	<.001 / .525	0.028 / 1.628	0.013 / .939

Different indices, from the following series: 1: a,b,c,d; 2: z,y,x,w,v,u; 3:)}][; 4: #@$; 5: -+=, within a column indicate significant differences (t-test; $P<0.001$) within each statistical model (Experimental design in table 2)

Table 4. Feed intake of birds of both sexes of two genotypes fed diets differing in amino acid pattern or P/E ratio. F-probabilities are shown according to five models of analysis.

			Feed intake (g feed) per bird per day during:		
Genotype	Sex	Diet	GP1 (0-21 d)	GP2 (22-43 d)	TGP (0d - 2500 g)
Com	male	1	$49.6^{d)-}$	$141.6^{c)+}$	$96.7^{b)-+}$
		2	$48.7^{cdx)\}\#-}$	$141.7^{cu)\#+}$	$99.8^{bx)\#+}$
		3	$45.6^{c)-}$	$143.9^{c)+}$	$99.3^{b)-+}$
		4	$49.9^{\#}$	$145.1^{\#}$	$98.6^{\#}$
		5	$47.5^{\#}$	$143.1^{\#}$	$100.5^{\#}$
		6	$48.2^{\#}$	$142.7^{\#}$	$100.1^{\#}$
		7	$47.6^{\#}$	$142.0^{\#}$	$99.6^{\#}$
		8	$47.3^{\#}$	$143.9^{\#}$	$100.3^{\#}$
		9	$49.2^{x)\}\#}$	$142.9^{u)\#}$	$97.1^{x)\#}$
		10	$50.0^{)}$	$143.1^{)}$	$97.7^{)}$
		11	$48.4^{)\}}$	$143.6^{)}$	$100.7^{)}$
		12	45.5^{-}	130.5^{-}	94.1^{-}
FC	male	1	30.5^{b}	99.3^{b}	78.1^{a}
		2	27.5^{abz}	91.3^{abyx}	76.2^{azy}
		3	26.9^{a}	84.3^{a}	76.1^{a}
		9	29.7^{z}	97.2^{x}	77.5^{y}
Com	female	2	43.6^{y}	124.2^{w}	95.9^{x}
		9	46.2^{yx}	130.6^{v}	99.2^{x}
FC	female	2	26.0^{z}	82.1^{z}	72.8^{z}
		9	28.6^{z}	88.1^{zy}	75.3^{zy}
Model	Factor		F-probabilities/sed		
1	P/E		<.001 / .603	<.001 / 1.470	0.869 / 1.127
	Genotype		<.001 / .492	<.001 / 1.200	<.001 / .920
	P/E * Genotype		0.116 / .853	<.001 / 2.078	0.061 / 1.593
2	AA-sup.		<.001 / .390	<.001 / .856	0.067 / .586
	Genotype		<.001 / .390	<.001 / .856	<.001 / .586
	Sex		<.001 / .390	<.001 / .856	0.003 / .586
	AA-sup. * Geno		0.300 / .552	0.208 / 1.210	0.176 / .829
	AA-sup. * Sex		0.101 / .552	0.131 / 1.210	0.004 / .829
	Genotype * Sex		0.001 / .552	0.002 / 1.210	0.109 / .829
	A * G * S		0.266 / .781	0.138 / 1.712	0.044 / 1.172
3	AA-sup.		0.020 / .498	0.635 / 1.627	0.936 / .981
	P/E ratio		<.001 / .610	0.715 / 1.992	0.080 / 1.201
	A * P		0.099 / .863	0.890 / 2.817	0.180 / 1.699
4	AA-sup.		0.008 / .728	0.742 / 2.085	0.193 / 1.356
5	Diet		<.001 / .883	<.001 / 2.415	0.005 / 1.477

Different indices, from the following series: 1: a,b,c,d; 2: z,y,x,w,v,u; 3:)}][; 4: #@$; 5: -+=, within a column indicate significant differences (t-test; $P<0.001$) within each statistical model (Experimental design in table 2)

Table 5. Nitrogen (N) intake of birds of both sexes of two genotypes fed diets differing in amino acid pattern or P/E ratio. F-probabilities are shown according to five models of analysis.

			Nitrogen intake (g ileal digestible N)/bird/day during:		
Genotype	Sex	Diet	GP1 (0-21 d)	GP2 (22-43 d)	TGP (0d - 2500 g)
Com	male	1	$1.43^{e][=}$	$3.82^{e]}+$	$2.65^{d]}+$
		2	$1.32^{dv}]\#@+=$	$3.62^{ev]}\#+$	$2.59^{dxw}]\#+$
		3	$1.15^{c]-}$	$3.36^{d]-}$	$2.36^{c]-}$
		4	$1.34^{\#@}$	$3.68^{\#}$	$2.54^{\#}$
		5	$1.29^{\#}$	$3.60^{\#}$	$2.57^{\#}$
		6	$1.32^{\#@}$	$3.72^{\#}$	$2.64^{\#}$
		7	$1.29^{\#}$	$3.66^{\#}$	$2.60^{\#}$
		8	$1.32^{\#@}$	$3.74^{\#}$	$2.65^{\#}$
		9	$1.39^{v][@}$	$3.79^{u)]\#}$	$2.62^{w)]\#}$
		10	$1.48^{[}$	$3.95^{]}$	$2.75^{]}$
		11	$1.29^{)}$	$3.59^{])}$	$2.56^{)}$
		12	1.26^{-+}	$3.62^{])+}$	2.61^{+}
FC	male	1	0.87^{b}	2.67^{c}	2.12^{b}
		2	0.74^{azy}	2.33^{by}	1.96^{abzy}
		3	0.67^{a}	1.96^{a}	1.80^{a}
		9	0.83^{x}	2.57^{x}	2.07^{y}
Com	female	2	1.18^{w}	3.17^{w}	2.47^{x}
		9	1.31^{v}	3.47^{v}	2.67^{w}
FC	female	2	0.70^{z}	2.10^{z}	1.87^{z}
		9	0.80^{yx}	2.34^{y}	2.02^{y}
Model	Factor		F-probabilities/sed		
1	P/E		<.001 / .0166	<.001 / .0384	<.001 / .0300
	Genotype		<.001 / .0136	<.001 / .0314	<.001 / .0245
	P/E * Genotype		0.010 / .0235	0.012 / .0543	0.277 / .0425
2	AA-sup.		<.001 / .0108	<.001 / .0224	<.001 / .0153
	Genotype		<.001 / .0108	<.001 / .0224	<.001 / .0153
	Sex		<.001 / .0108	<.001 / .0224	0.003 / .0153
	AA-sup. * Geno		0.944 / .0153	0.817 / .0317	0.748 / .0217
	AA-sup. * Sex		0.138 / .0153	0.201 / .0317	0.003 / .0217
	Genotype * Sex		<.001 / .0153	0.001 / .0317	0.251 / .0217
	A * G * S		0.287 / .0216	0.163 / .0448	0.032 / .0307
3	AA-sup.		<.001 / .0141	<.001 / .0428	<.001 / .0261
	P/E ratio		<.001 / .0172	<.001 / .0524	<.001 / .0320
	A * P		0.055 / .0243	0.652 / .0742	0.059 / .0452
4	AA-sup.		<.001 / .0201	0.017 / .0537	0.042 / .0352
5	Diet		<.001 / .0248	<.001 / .0640	<.001 / .0393

Different indices, from the following series: 1: a,b,c,d; 2: z,y,x,w,v,u; 3:)}][; 4: #@$; 5: -+=, within a column indicate significant differences (t-test; $P<0.001$) within each statistical model (Experimental design in table 2)

compared to the other supplemented diets; however not compared to the basal diet (2). The comparison of diets 1,2,3 and the control diet revealed in GP1 a significant effect. The control diet resulted in a growth rate intermediate between the medium and low P/E ratio; significantly ($P < 0.001$) different from birds fed the high P/E ratio diet.

Feed/Nitrogen intake

Tables 4 and 5 show feed and nitrogen intake, respectively, including the results of the statistical analysis based on the five models mentioned above. According to model [1], male FC birds distinctly had a lower feed and nitrogen intake than Com birds in all considered periods. In young birds (0-21 d) a lower P/E ratio resulted in a lower feed as well as nitrogen intake while in older birds (22-43 d) P/E ratio affected feed intake only in FC birds and not in Com birds. Similar, in GP2 N intake was more decreased with decreas P/E ratio in FC birds than in Com birds. When TGP was considered feed intake was similar for al three P/E ratio's and consequently nitrogen intake was lowered with lower P/E ratio.

Analysis according to model [2] showed, that Com birds ate more and thus had a higher N-intake than FC birds in all periods. In general, male birds have a higher feed- and N-intake than female birds. However, there were some interactions between sex and either genotype or amino acid supplementation. In GP1 differences in feed- and nitrogen-intake between male and female birds were small or absent in FC birds. In GP2 nitrogen intake was less affected by sex in FC birds than in Com birds. When TGP was considered, the interaction between sex and amino acid supplementation was found in a positive effect of amino acid supplementation on feed- and nitrogen-intake in female birds whereas in male birds there was no effect.

According to the third model in GP1 there was a higher feed- and nitrogen-intake from supplemented diets while lower P/E ratio resulted in lower feed- and nitrogen-intake. During GP2 and when TGP was considered, no effects on feed intake were apparent. However, nitrogen intake was affected by supplementation as well as by P/E ratio, according to the relative nitrogen content of the diet.

F-test according to the fourth model revealed that in GP1 feed as well as nitrogen intake was affected by amino acid supplementation while in GP2 and when TGP was considered only nitrogen intake was affected. The total supplemented diet resulted in highest feed- and nitrogen-intake.

Table 6. Feed conversion ratio of birds of both sexes of two genotypes fed diets differing in amino acid pattern or P/E ratio. F-probabilities are shown according to five models of analysis.

			Feed conversion ratio (g growth)/(g feed) during:		
Genotype	Sex	Diet	GP1 (0-21 d)	GP2 (22-43 d)	TGP (0d - 2500 g)
Com	male	1	$1.39^{a,),-}$	$1.83^{c,),-,+}$	$1.69^{b,),},-$
		2	$1.43^{b,y,l,@,+}$	$1.80^{c,x,),\#,-,+}$	$1.72^{b,yx,),@\$,-}$
		3	$1.47^{c,l,=}$	$1.85^{c,),+}$	$1.78^{c,l,+}$
		4	$1.39^{\#}$	$1.82^{\#}$	$1.69^{\#,@}$
		5	$1.43^{@}$	$1.79^{\#}$	$1.72^{@\$}$
		6	$1.43^{@}$	$1.83^{\#}$	$1.75^{\$}$
		7	$1.43^{@}$	$1.83^{\#}$	$1.75^{\$}$
		8	$1.43^{@}$	$1.79^{\#}$	$1.74^{\$}$
		9	$1.36^{z,),\},\#}$	$1.80^{x,),\#}$	$1.67^{y,),\#}$
		10	$1.34^{)}$	$1.83^{)}$	$1.68^{)}$
		11	$1.39^{)}$	$1.84^{)}$	$1.74^{)}$
		12	$1.41^{-,+}$	1.77^{-}	1.72^{-}
FC	male	1	1.37^{a}	1.55^{a}	1.58^{a}
		2	$1.43^{b,y}$	$1.53^{a,z}$	$1.60^{a,z}$
		3	1.48^{c}	1.62^{b}	1.69^{b}
		9	1.34^{z}	1.55^{z}	1.59^{z}
Com	female	2	1.50^{x}	1.91^{w}	1.91^{w}
		9	1.42^{y}	1.98^{v}	1.92^{w}
FC	female	2	1.46^{y}	1.61^{y}	1.74^{x}
		9	1.37^{z}	1.62^{y}	1.76^{x}
Model	Factor		F-probabilities/sed		
1	P/E		<.001 / .00678	<.001 / .01052	<.001 / .00880
	Genotype		0.921 / .00554	<.001 / .00859	<.001 / .00718
	P/E * Genotype		0.048 / .00959	0.032 / .01488	0.220 / .01244
2	AA-sup.		<.001 / .00508	0.002 / .00836	0.160 / .00762
	Genotype		<.001 / .00508	<.001 / .00836	<.001 / .00762
	Sex		<.001 / .00508	<.001 / .00836	<.001 / .00762
	AA-sup. * Geno		0.037 / .00719	0.190 / .01183	0.155 / .01078
	AA-sup. * Sex		0.416 / .00719	0.093 / .01183	0.006 / .01078
	Genotype * Sex		<.001 / .00719	<.001 / .01183	<.001 / .01078
	A * G * S		0.911 / .01017	0.032 / .01673	0.505 / .01524
3	AA-sup.		<.001 / .00472	0.990 / .01142	<.001 / .00736
	P/E ratio		<.001 / .00579	0.025 / .01398	<.001 / .00901
	A * P		0.128 / .00818	0.803 / .01978	0.173 / .01274
4	AA-sup.		<.001 / .00746	0.359 / .02201	<.001 / .01335
5	Diet		<.001 / .00805	0.006 / .01842	<.001 / .01138

Different indices, from the following series: 1: a,b,c,d; 2: z,y,x,w,v,u; 3:)}][; 4: #@$; 5: - + =, within a column indicate significant differences (t-test; $P<0.001$) within each statistical model (Experimental design in table 2)

There was a significant effect of diet in the fifth model on feed- and nitrogen-intake. During GP2 and when TGP was considered feed intake of the control diet was lower than each of the test diets. Nitrogen intake from the control diet was intermediate between low and medium P/E diets.

Feed conversion ratio (FCR)

Feed to gain ratio (FCR) is given in table 6.

According to model [1], during GP1 there was a significant ($P<0.05$) interaction between genotype and P/E ratio. In FC birds the difference between high and low P/E ratio was larger than in Com birds. The main effect of P/E ratio ($P<0.001$) can be described as an increase in FCR with decreasing P/E ratio. In phase 2 there was a clear effect of genotype ($P<0.001$) resulting in a much lower feed conversion ratio for FC birds. The significant ($P<0.05$) interaction between genotype and P/E ratio can be found in a difference in FCR between medium and low P/E ratio in FC birds whereas in Com birds this effect was absent. When TGP is considered then no interaction between genotype and P/E ratio is detectable. The main effect of P/E ratio ($P<.001$) was that a decrease in P/E ratio results in an increase in FCR. At each P/E ratio the FC-genotype had a lower FCR than the Com-genotype.

According to model [2], there was a significant ($P<0.001$) interaction between genotype and sex with regard to FCR in each period. In GP1 male and female FC birds had the same FCR whereas in Com birds FCR was affected by sex. In GP2 the effect of genotype was larger in female than in male birds. For TGP the effect of genotype on FCR was also larger in female than in male birds. In GP2 there was a significant ($P<0.05$) interaction between amino acid supplementation, genotype and sex: In female Com birds supplementation with the complete amino acid mixture resulted in a significant increase in FCR whereas in other genotypes/sexes supplementation resulted in no effect. When TGP was considered there was a significant ($P<0.01$) interaction between AA-supplementation and sex. In male chicks AA-supplementation decreased FCR whereas in female chicks AA-supplementation had no effect on FCR. The main effect of amino acid supplementation differed over the respective periods. In GP1 FCR was decreased whereas in GP2 FCR was increased resulting in the absence of an effect of AA-supplementation on FCR over TGP. The effects of genotype and sex were more similar between periods: In all periods FC birds had a lower FCR compared to Com birds and female birds had in general a higher FCR compared to male birds.

In model [3], where the effect of AA-supplementation and P/E ratio was tested in male Com birds, no interactions were detected. In GP1 and over TGP AA-

Table 7. Nitrogen efficiency of birds of both sexes of two genotypes fed diets differing in amino acid pattern or P/E ratio. F-probabilities are shown according to five models of analysis.

			Nitrogen efficiency (N in body accretion)/(ileal digestible N-intake)		
Genotype	Sex	Die	GP1 (0-21 d)	GP2 (22-43 d)	TGP (0d - 2500 g)
Com	male	1	.624$^{a,),-}$	.540$^{a,),-}$	.562$^{a,),-}$
		2	.621$^{a,zyx,),\#@,-}$	.572$^{a,y,),\#,-}$	.623$^{b,x,),@,+=}$
		3	.654$^{a,),-}$	.574$^{a,),-}$	.627$^{b,),=}$
		4	.654$^{@}$	.550$^{\#}$	.577$^{\#@}$
		5	.617$^{\#@}$	.591$^{\#}$	.603$^{\#@}$
		6	.615$^{\#@}$	.552$^{\#}$	.568$^{\#}$
		7	.620$^{\#@}$	.557$^{\#}$	.597$^{\#@}$
		8	.604$^{\#}$	.567$^{\#}$	.596$^{\#@}$
		9	.628$^{zyx,),\#@}$	.564$^{y,),\#}$	.580$^{yx,)),\#@}$
		10	.626$^{)}$	.531$^{)}$	.556$^{),\#}$
		11	.651$^{)}$	.575$^{)}$	.597$^{))}$
		12	.615^{-}	.532^{-}	.582^{-+}
FC	male	1	.654^{a}	.665^{b}	.679^{c}
		2	.675^{a,xw}	.689^{b,x}	.694^{cd,w}
		3	.673^{a}	.703^{b}	.721^{d}
		9	.689^{w}	.668^{x}	.697^{w}
Com	femal	2	.603^{z}	.524^{zy}	.525^{zy}
		9	.610^{zy}	.471^{z}	.487^{z}
FC	femal	2	.650^{zyxw}	.669^{x}	.635^{x}
		9	.6642^{xw}	.637^{x}	.604^{x}
Model	Facto		F-probabilities/sed		
1	P/E		0.110 / .01122	0.007 / .01079	<.001 / .00714
	Genotype		0.001 / .00916	<.001 / .00881	<.001 / .00583
	P/E * Genotype		0.304 / .01587	0.858 / .01525	0.014 / .01010
2	AA-sup.		0.128 / .00695	0.002 / .00861	0.001 / .00845
	Genotype		<.001 / .00695	<.001 / .00861	<.001 / .00845
	Sex		0.004 / .00695	<.001 / .00861	<.001 / .00845
	AA-sup. * Geno		0.617 / .00983	0.805 / .01217	0.131 / .01195
	AA-sup. * Sex		0.996 / .00983	0.108 / .01217	0.414 / .01195
	Genotype * Sex		0.664 / .00983	0.014 / .01217	0.248 / .01195
	A * G * S		0.995 / .01390	0.321 / .01722	0.268 / .01690
3	AA-sup.		0.787 / .00787	0.556 / .00954	<.001 / .00650
	P/E ratio		0.011 / .00964	0.006 / .01168	<.001 / .00797
	A * P		0.848 / .01363	0.897 / .01652	0.088 / .01126
4	AA-sup.		<.001 / .00924	0.407 / .01926	0.004 / .01314
5	Diet		0.078 / .01483	0.011 / .01331	<.001 / .00941

Different indices, from the following series: 1: a,b,c,d; 2: z,y,x,w,v,u; 3:)}][; 4: #@$; 5: -+=, within a column indicate significant differences (t-test; $P<0.001$) within each statistical model (Experimental design in table 2)

supplementation resulted in a significant ($P<0.001$) reduction in FCR. A decrease in P/E ratio resulted in a higher FCR.
The effect of different amino acid supplementations on FCR was found significant ($P<0.001$) in GP1 and also when TGP was considered. In GP1 the Met-supplemented diets (4,9) were significant (t-test; $P<0.001$) different from the basal diet whereas the other supplementations had no effect. When TGP was considered only the diet where all amino acids were supplemented (9) was different from the basal diet. The individual supplementations were not different from the basal diet while the extra Met diet resulted in a significant lower FCR compared to diets with other supplementations.
The comparison of the three P/E ratio's to the control diet revealed that in all periods the control diet resulted in a significant (t-test; $P<0.001$) lower FCR compared to the low P/E ratio.

Nitrogen efficiency

The overall efficiency with which ileal digested nitrogen is used for body nitrogen accretion is given in table 7. Significant effects in the respective periods according to the five previous mentioned models are mentioned below.
Analysis according to the first model revealed that in all periods N-efficiency of FC birds was higher ($P\leq0.001$) than that of Com birds. In GP2 the highest P/E ratio resulted in a significant ($P<0.01$) lower N-efficiency compared to medium and low P/E ratio. When TGP is considered then an interaction between genotype and P/E ratio was significant ($P<0.05$). In FC birds all three P/E ratio's resulted in significant different nitrogen efficiencies whereas in Com birds only the high P/E ratio resulted in a lower N-efficiency compared to the other two P/E ratio's.
In the second model only for GP2 an interaction was found. In FC birds nitrogen-efficiency of female birds was more close to nitrogen efficiency of male birds than in Com birds. FC birds in general had a higher nitrogen efficiency compared to com birds. Male and female birds displayed the same contrast. In GP2 and when TGP is considered there was also an effect of AA-supplementation on N-efficiency. Supplementation of the diet with a mixture of Met, Arg, Glu and Gly resulted in a lower N-efficiency ($P<0.01$).
The effect of P/E ratio, as tested in model [3], was significant in all periods. In GP1, the low P/E ratio resulted in an improvement ($P<0.05$) of N-efficiency. In GP2 ($P<0.01$) and over TGP ($P<0.001$) N-efficiency of birds fed the high P/E ratio was lower than when the other two P/E ratio's were fed. When TGP is considered supplementation with Met + Arg + Glu + Gly resulted in a lower N-efficiency.

Table 8. Amino acid pattern (amino acid as g/100 g total analyzed amino acids) of 21 days old male com birds fed high, medium or low P/E diets compared with reference amino acid pattern.

	Dietary P/E ratio					Reference
AA	High	Medium	Low	sed	Mean	pattern[1]
Cys	1.37	1.40	1.33	.045	1.37	1.94
Asp	9.43	9.46	9.44	.053	9.44	8.90
Met	2.11	2.12	2.10	.034	2.11	2.41
Thr	4.63	4.59	4.63	.048	4.62	4.31
Ser	5.17	5.17	5.15	.027	5.16	5.01
Glu	14.7	14.7	14.7	.079	14.7	13.2
Pro	5.60	5.45	5.53	.093	5.53	5.90
Gly	7.97	7.77	7.77	.139	7.84	8.59
Ala	6.58	6.56	6.62	.046	6.58	7.64
Val	5.54	5.56	5.59	.037	5.56	5.91
Ile	4.66	4.71	4.69	.040	4.69	4.44
Leu	7.88	8.00	8.03	.037	7.97	7.34
Tyr	3.17	3.22	3.18	.034	3.19	3.18
Phe	4.11	4.12	4.12	.029	4.12	4.26
His	3.57	3.59	3.54	.068	3.57	3.56
Lys	6.69	6.78	6.80	.107	6.76	6.59
Arg	6.84	6.80	6.79	.051	6.81	6.76

[1] Pattern in 21 day old male Com broiler chicks as determined by ten Doeschate et al. (1991).

Analysis according to model [4] revealed that in GP1 and over TGP there existed a significant ($P<0.001$ and $P<0.004$, respectively) effect of diet. In GP1 Student's t-test ($P<0.001$) detected no effect relative to the basal diet. The Met-supplemented diet resulted in a higher N-efficiency compared to the diet supplemented with Glu + Gly. The N-efficiency over TGP was, compared to the basal diet, lower in the Glu-supplemented group. In GP2 no significant effect ($P=0.407$) of amino acid supplementation was found.
The effect of diet on N-efficiency when the three P/E ratio's and the control diet were compared was significant in GP2 ($P<0.05$) and when TGP is considered ($P<0.001$). Nitrogen efficiency of the control diet was in GP2 lower than all three test diets but when TGP is considered the control diet was intermediate between the high and medium P/E ratio test diet.

Amino acid composition

In three week old male Com birds amino acid composition was determined when three levels of dietary P/E ratio were fed. In table 8 the whole body amino acid contents, expressed as g per 100 g total analyzed amino acids, of these groups is given, combined with data from a previous experiment (ten Doeschate et al., 1991). Statistical analysis showed that only leucine was significantly ($P=0.005$) affected by P/E ratio. Relative Leu-content in the body increased with lower dietary P/E ratio.

Discussion

P/E ratio

Several performance parameters were affected by P/E ratio. It was shown that FC birds were more sensitive to differences in P/E ratio than Com birds which is in accordance with the conclusion reached by Leenstra (1991). Most performance parameters were affected in a negative manner by a decrease in P/E ratio. At the low P/E ratio protein intake thus limited protein deposition. The low observed protein intake is caused by the regulation of dietary intake by dietary energy content. In contrast with limited protein deposition, N-efficiency was raised with lower P/E ratio. The higher N-efficiency observed with lower P/E ratio is in accordance with the findings of Heger & Frydrich (1991) in rats. Probably, this

effect is constrained to a certain range of P/E ratios. In pigs, Bikker (1994) observed protein and energy dependent phases in protein deposition. This implies that an optimum P/E ratio should exist. The higher nitrogen efficiency at lower P/E ratio's corresponds with lower growth rate and higher FCR. The lower performance is compensated for by a lower N-intake. The better nitrogen efficiency observed at 43 days of age is still observed in birds slaughtered at similar slaughter weight which illustrates that the higher efficiency displayed at 43 days of age is not caused by a simple effect of lower body weight. In GP1 the contrast in N-efficiency caused by dietary P/E ratio can be found in the contrasts between medium or high P/E and low P/E. This is an indication that during this period the medium and high P/E ratio's are too high. For GP2 the main contrast can be found between low or medium and high P/E ratio. When TGP is considered also the contrast between low or medium and high P/E ratio can be considered the main contrast. For GP2 thus only the high P/E ratio can be considered as being too high. It can be concluded that both the low and the medium P/E ratio will result in an equally high N-efficiency. Because growth rate and FCR showed a regular increase with P/E ratio it would be optimal to use our medium P/E ratio. However, the optimal P/E ratio in practice is more a economical than a physiological decision. The optimum in practice will depend on the relative cost of lower growth rate, higher FCR and lower nitrogen efficiency.

Genotype

In general, FC birds had a lower growth performance combined with a better FCR and N-efficiency than Com birds. Part of the better efficiency of the FC birds could be explained by the higher efficiency in digestion as was shown by ten Doeschate et al.(1993). Mean amino acid digestibility was approximately 3 % and metabolisability 2 % higher in FC birds than in Com birds (ten Doeschate et al., 1993). Nitrogen efficiency over TGP (table 7) can be calculated to be approximately 15-20 % higher in FC than in Com birds. The higher digestibility as measured in FC birds can thus explain a part of the higher efficiency in FC birds. The higher N-efficiency observed is, at least partly, also caused by a more efficient deposition of absorbed nitrogen. This more efficient nitrogen deposition can be caused by the difference in growth pattern as observed previously (ten Doeschate et al., 1995). The continuous increase in growth rate with age in FC birds will, especially in older birds, result in more efficient growth than the declining increase in growth rate observed in Com birds. Maintenance requirements for amino acids are directly

related to length of the growing period required to attain the desired slaughter weight. Due to lower growth rate a larger part of total daily requirements would be maintenance requirements. As FC birds use more days to reach a given slaughter weight it would be expected that they would require a different dietary amino acid pattern to compensate for the relative higher maintenance requirements. For this reason in model [2] a significant interaction between genotype and amino acid supplementation was to be expected. However, no significant interaction between genotype and amino acid supplementation was found. This supports the hypothesis of ten Doeschate et al. (1995) which states that carcass amino acid pattern can be used as a reliable reference for a practical dietary amino acid pattern.
The possible interactions between genotype and dietary P/E ratio were a second reason why the FC bird was used in the present study besides the Com bird. Based on growth rate and FCR it has been suggested that FC birds react more favourable to an increase in protein as growth selected birds (Leenstra et al., 1986). For this reason, it was expected that optimal nitrogen-efficiency would be reached at a higher P/E ratio in FC birds than in Com birds. There were several interactions between genotype and dietary factors. For nitrogen efficiency such an interaction was only found between genotype and P/E ratio when TGP was considered. It was shown that in FC birds all both steps of lower P/E ratio resulted in a higher N-efficiency whereas in Com birds only the step from high to medium P/E ratio resulted in a significant improvement. If N-efficiency is used as response-parameter then FC birds perform best on low P/E ratio which seems to be in contrast with Leenstra et al. (1986). However, Leenstra et al. (1986) used growth rate and FCR as response parameters and with regard to these parameters also in our experiment the FC-bird reacts favourable to high P/E ratio's. It can be concluded that, in order to judge efficiency, only direct efficiency traits should be used.

Sex

The effect of sex is quite clear: males show a higher growth rate, lower FCR and a higher nitrogen efficiency. In FC birds sometimes the difference between male and female chicks was less obvious or even absent resulting in interaction between genotype and sex. The higher efficiency of males can not be explained by a higher digestibility of the diet since digestibility is higher in female birds (ten Doeschate et al., 1993). The observed sex-difference is thus a metabolic effect. The amino acid pattern of body accretion, used for diet formulation in this experiment, was determined in male broilers (ten Doeschate et al., 1995). It was

shown that whole body amino acid composition (and thus amino acid composition of body accretion) of female chicks differed from that of male chicks. Previously (ten Doeschate et al., 1995) it was stated that we expected not a great decrease in efficiency of nitrogen utilization in female broiler chicks if the 'male' pattern was used as basis for diet formulation. The reason for this assumption was that differences between sexes were not very substantial. In general, female chicks contained more non-essential and less essential amino acids. In the present experiment, female birds showed much lower N-efficiency than male birds when a 'male' pattern was used as basis for diet formulation. However, observed differences in N-efficiency were much larger than could be expected based on differences in amino acid pattern between male and female chicks. It could be suggested that optimal dietary P/E ratio for female chicks differs from that for male chicks but we do not have experimental data for this assumption. Based on the present results we conclude that, when given similar diets, nitrogen efficiency in female broiler chicks is much lower than in male broiler chicks; especially when compared at equal slaughter weight. With respect to optimal nitrogen efficiency in broiler production systems it would be advisable to slaughter female chicks at lower body weights than male chicks.

Amino acid supplementation

Supplementation with Arg or Met was tested because it was expected that if Met or Arg are supplied at a level according to body accretion this would lead to a relative undersupply because of metabolic functions. Non-essential amino acids (Glu, Gly) were added because at low P/E ratio the total nitrogen intake might become limiting for maximal performance (Holsheimer & Janssen, 1991; Moran et al., 1992). Since Glu is interconvertible with Ala, Asp and Pro while Gly is interconvertible with Ser the combination of Glu and Gly was used as a complete supplementation of non-essential amino acids. Only a few contrasts within the different amino acid supplementations with regard to performance parameters were found significant. Addition of Met had a positive effect on growth rate and FCR in GP1 which was reflected in FCR when TGP was considered. For nitrogen efficiency in GP1 Met-supplemented diets resulted in higher N-efficiency than (Glu + Gly)-supplemented diets. When TGP was considered, the Glu-supplemented diet negatively affected N-efficiency whereas Met-supplementation had no effect. From these results we conclude that the level of Arg in a dietary pattern according to body accretion is sufficient for both growth and maintenance requirements. Met-

supplementation might improve growth rate without negative effects on nitrogen efficiency. Supplementation with non-essential amino acids to a carcass amino acid pattern is not only not necessary but has negative effects on nitrogen efficiency.

Supplementation with a mixture of amino acids (Met + Arg + Glu + Gly) is, based on the results of the individual amino acids, not expected to have positive effects on nitrogen efficiency. It did have a positive effect on growth rate and FCR in some periods. In GP2, growth rate of FC birds was more stimulated than growth rate of Com birds. The extra nitrogen intake with supplemented diets resulted in lower nitrogen efficiency when supplemented diets were compared to un-supplemented diets. When the three P/E ratio's were supplemented it was shown that supplementation had a larger effect on growth rate at low P/E level than at high P/E level implying that at the high P/E level maximal growth rate was already attained or energy intake limited growth rate and thus supplementation was not able to increase growth rate. The effect of AA-supplementation was especially seen in young birds. This indicates that young birds have a capacity for higher growth rate than that attained at the high P/E level. Thus, in young birds fed the un-supplemented diets, protein intake was limiting protein gain.
The magnitude of the effect on growth rate of AA-supplementation is similar to Met-supplementation alone. This suggests that it is a singular effect of Met-supplementation. The level of Met in a diet according to the AA pattern of body accretion might thus be sub-optimal for maximal growth rate but not for maximal nitrogen efficiency.

Conclusions

It is shown that utilisation of the amino acid pattern of body accretion can be used as basis for dietary amino acid pattern results in high N-efficiency without necessary supplementation of methionine or arginine. There were no indications for differences in Met or Arg requirements in two genetic stocks. When the amino acid pattern of body accretion is used as basis for diet formulation there is no relative deficiency of non-essential amino acids as was shown in birds fed diets supplemented with Glu and Gly. P/E ratio affects N-efficiency and performance parameters. Optimal P/E ratio should be determined by economical evaluation.

In general, it has been shown that dietary measures may influence nitrogen efficiency. However, differences in genotype (Com vs FCR) resulted in a wider range of nitrogen efficiency. A general conclusion is that a higher growth performance is associated with a lower N-efficiency. Maximal growth rate and

maximal nitrogen efficiency can thus not be attained at the same time. It is important to realize that the results obtained with AA-supplementation show that requirements for maximal N-efficiency differ from those for maximal production. It would be advisable to evaluate requirement studies with a certain objective in order to attain not only requirements for maximal growth rate but also requirements for maximal N-efficiency. To reach maximal (or optimal) nitrogen efficiency, one has to be prepared to be satisfied with lower performance. The economic feasible nitrogen efficiency thus depends on the price of nitrogen excretion relative to the cost of increasing nitrogen efficiency (cost of feed, extra chicks, longer growing period etc.). The price of nitrogen excretion and cost of feed can be partly influenced by governmental policy with the aim to reduce environmental pollution. This study indicates that, if the nitrogen efficiency of male Com birds fed the control diet (12) during TGP is taken as basis, nitrogen efficiency could be increased with 7.5 % if the low P/E diet is used and with 23 % if the low P/E ratio is fed to FC birds. The relative nitrogen excretion, to reach similar slaughter weights, is given for all groups in table 9. It can be seen that the present dietary changes affect nitrogen excretion less than genetic improvement. Nevertheless, it is shown that also in geneticly more efficient birds dietary changes still affect nitrogen excretion. Both methods of improvement of nitrogen efficiency can thus be considered complementary.

Table 9. Relative nitrogen excretion of all groups for the entire growth period until equal weight was reached (TGP). (% relative to male Com birds fed diet 12)

Genotype	Sex	Diet	Relative nitrogen excretion (percentage of male Com birds fed diet 12) 0 d - 2500 g
Com	male	1	105
		2	90
		3	89
		4	102
		5	95
		6	104
		7	96
		8	97
		9	100
		10	106
		11	96
		12	100
FC	male	1	77
		2	73
		3	67
		9	73
Com	female	2	114
		9	123
FC	female	2	87
		9	95

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Chapter 6

NITROGEN EFFICIENCY OF MALE BROILER CHICKS FED DIETS DIFFERING IN AMINO ACID PATTERN, NON-ESSENTIAL AMINO ACID AND THREONINE CONTENT.

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To be submitted

Summary

The efficiency of nitrogen deposition can be influenced by dietary nitrogen supply. In the experiments described, several hypotheses concerning the effect of dietary amino acid pattern on performance parameters such as growth feed conversion ratio and N-efficiency are tested.

In the first experiment a comparison is made between a practical diet and experimental diets based on either literature values or the amino acid pattern of body accretion plus some adaptations of these patterns. It was shown that the amino acid pattern of body accretion can be used as basis for calculation of amino acid requirements. There were no differences in response between a diet based on literature or a diet based on the amino acid pattern of body accretion. The practical diet resulted in a lower N-efficiency, higher growth rate and lower FCR. In the second growth phase (15-35 d of age) there was a positive effect of extra methionine (relative to the pattern of body accretion) on growth and FCR without negative effect on N-efficiency. This implies that in this growth phase dietary methionine level should be higher than that based on the level of body accretion.

In the second experiment was tested whether it was possible to promote utilisation of non-essential amino acids formed in the obligatory breakdown of essential amino acids for nett protein synthesis by lowering the dietary non-essential amino acid level. Hereto nine diets with varying levels of glutamic acid + aspartic acid + alanine (NEAA) were fed to male broiler chicks. It was shown that with lower NEAA levels growth rate was lowered, FCR was higher but N-efficiency was higher. Maximal growth rate and maximal N-efficiency can thus not be reached simultaneously.

The third experiment was based on the theory that high NEAA excess will lead to extra uric acid production which will lead to an increase in glycine requirement. Since glycine can be formed from threonine, threonine requirement might be affected by NEAA level. In an experiment with three threonine and three NEAA levels was shown that at high NEAA level the effect of low threonine level was more severe than at low NEAA level. This supports the hypothesis that the requirement of threonine is determined not only by the requirement for body accretion but also by a requirement for glycine production to excrete excess nitrogen.

Introduction

The efficiency of conversion of nitrogen in the diet of animals into nitrogen in the desired animal products is of major importance with regard to environmental nitrogen pollution. In poultry, excess absorbed nitrogen is excreted mainly as uric acid which can be easily transformed into ammonia by microbial action. The excretion of uric acid per unit product should thus be diminished if the objective is to diminish this contribution to ammonia pollution. This objective will be reached if the supply of apparent ileal nitrogen compounds is maximally adjusted to the requirement. Amino acid pattern of the absorbed nitrogen should mirror the requirement without excesses or shortages of amino acids. In this way only obligatory nitrogen losses contribute to uric acid excretion. For broiler chicks, the amino acid profile of body accretion can be used as a basis for establishing amino acid requirements. This approach was tested in an experiment in which the amino acid pattern according to body accretion was used in diets with three protein:energy ratio's, fed to birds of two genotypes (ten Doeschate et al., 1995b). The results indicated that following this approach efficiencies of total nitrogen retention of 63 % of ileal digestible nitrogen intake can be reached. In literature, amino acid requirements are either calculated from several requirement studies (Baker & Han, 1994) or are calculated in a factorial approach in which besides a net requirement for growth an estimated maintenance part is included (Hurwitz et al., 1978, 1980; Fisher, 1993). The composition of net growth can then be derived from amino acid composition of whole body accretion or from amino acid composition of separate tissues (muscle, skin, feathers etc). The composition of the maintenance part however is disputable. At first a correct definition of maintenance is required. For energy, maintenance requirement is defined as the amount of energy intake required to maintain the body in a steady state. It can be measured by an extrapolation of the energy balance to zero energy balance from balance data determined at a variety of energy intake levels. A similar definition would be possible for amino acids but it would be hard to determine this requirement experimentally for indicidual amino acids. Schreurs et al. (1993) avoided the term 'maintenance' requirements by introduction of the term 'non-productive' requirements. These non-productive requirements comprise the needs for specific functions (histidine for histamine-production, methionine as methyl donor) and the needs to compensate unavoidable losses related to metabolic degradation of amino acids.

These non-productive requirements may differ with the production level and are thus not fixed like maintenance requirements are supposed to be. The requirement of amino acids for production of digestive enzymes and other endogenous losses is provided for by calculation of available amino acids based on apparent ileal digestibility. This leaves in broilers only a relative low 'non-productive' requirement.

The N released from catabolized amino acids is, in poultry, converted to uric acid and excreted. The precursors of uric acid are glutamine, aspartic acid and glycine (Stryer, 1985). This means that all nitrogen to be excreted passes the stage of non-essential amino acids before excretion. If these non-essential amino acids, instead of being disposed of as uric acid, could be utilized for net protein deposition, N-efficiency would improve. This could be tested by lowering the content of non-essential amino-N in a diet when the dietary pattern of essential amino acids was adapted to the pattern of body accretion. Only the normal products of transamination processes (glutamine/glutamic acid (Glu) + alanine (Ala) + aspartic acid (Asp)) should be lowered.

Furthermore, the glycine required for uric acid formation is incorporated entirely into uric acid. Threonine can serve as a precursor for glycine, either directly or through serine (Bender, 1985). This means that when a relative large excess of nitrogen is to be excreted and when no extra glycine or serine is available there may be an increased non-productive threonine requirement.

This paper describes three experiments in which nitrogen efficiency in broilers over 0-35 days of age was measured. In the first experiment, three amino acid patterns and some variations are compared. The second experiment investigates the effect lowering of non-essential amino acid content at a constant level of essential amino acids to study the hypothesis that non-essential amino acids can be re-utilized. In the third experiment three levels of non-essential amino acids are combined with three levels of threonine to investigate whether the level of excess nitrogen affects the (non-productive) threonine requirement.

Materials and methods

Birds and environment

In total 2070 male birds from a commercial broiler hybrid (HYBRO, Euribrid, Boxmeer, the Netherlands) were raised in litter floor pens (75*97 cm).

Per pen 15 birds were placed. In total, 144 pens were available in 9 separate rooms. The whole experiment comprised three separate experiments. The eight treatments from the first experiment were, in three rooms, per room randomly allocated to two pens per treatment. The 15 treatments of the second and third experiment were per room randomly allocated to one pen per treatment in the remaining six rooms. In these six rooms one pen was left empty. The environmental temperature was decreased gradually from 32 °C for one-d-old chickens to 19 °C at 5 weeks of age. For the first 3 d, there was continuous light. To prevent leg disorders and to stimulate a regular feed intake, the birds where kept under a lighting regime in 1 h light alternating with 2 h darkness from 3 d of age. Unless stated otherwise, the birds had access to food and water *ad libitum*. Day-old chicks were vaccinated against Newcastle Disease (NCD) and Infectious Bronchitis. Birds were also vaccinated against bursal (Gumboro) disease at 17 days of age and at 21 days of age against NCD.

Diets

Two growth phases were used, 0-14 and 15-35 days of age (ten Doeschate et al., 1991). Diets for each growth phase were composed according to a similar principle. In total there were 23 diets. Diets 1,2 and 4-8 were based on a basal mixture supplemented with amino acids. Also diets 9-23 were composed in a similar way. Per growth phase, all diets were isocaloric and had a similar apparent ileal digestible lysine content. The only difference between diets was thus the amino acid content relative to lysine. All synthetic amino acids used, except Met and Gly, were pharmaceutical grade L-amino acids purchased from ORFFA Netherlands Food BV. The individual diets are described below with abbreviations used for denomination in brackets:

1) The amino acid pattern was based on the amino acid pattern of body accretion in the subsequent growth phases as determined by ten Doeschate et al. (1995a) (tD)
2 The amino acid pattern was based on Baker & Han, 1994 (Baker)
3) This diet was composed to constitute a diet similar to practical diets in use in the Netherlands in 1994. (Prac)
4) Diet 1 with a lower level of histidine (His); intermediate between diet 1 and 2. This diet was designed to test whether the high level of His found in body accretion (ten Doeschate et al., 1995a) was necessary for optimal performance. (1-His)

Table 1. Composition of diet 1, 2, 3 and 9 in both growth phases (0-14 and 15-35 d of age). The other experimental diets were composed by exchange of tapioca and soya oil by synthetic amino acids. (g/kg)

	1A	2A	3A	9A	1B	2B	3B	9B
Maize	182.46	182.46	193.28	396.97	160.74	160.74	140.0	400.0
Soyabeans, heated	27.77	27.77	116.0	0	111.36	111.36	110.0	0
Maize glutenfeed	0	0	0	100.0	0	0	0	100.0
Wheat	150	150	150	0	130.0	130.0	181.74	0
Peas	132.73	132.73	80	64.50	56.14	56.14	50.0	59.31
Maize glutenmeal	17.23	17.23	0	0	23.19	23.19	0	0
Meat meal	52.5	52.5	40	53.0	52.5	52.5	40.0	52.5
Sunflower meal, solv. extr.	75.0	75.0	0	19.25	75.0	75.0	0	11.63
Soyabean meal, solv. extr.	29.15	29.15	170	0	38.18	38.18	170.0	0
Tapioca	178.57	177.38	152.66	196.8	192.91	191.94	200.0	192.01
Fish meal	37.02	37.02	30.0	75.0	4.68	4.68	30.0	67.96
Soya oil	41.5	41.5	38.88	24.60	45.0	45.0	54.10	41.0
Sugar	26.96	26.96	0	0	65.0	65.0	0	10.12
L-Arg-HCl	1.16	1.78	0	3.92	0.26	2.16	0	3.40
Gly	5.67	5.67	0	7.09	3.68	3.68	0	5.22
L-His	2.2	0	0	2.32	2.46	0	0	2.67
L-Ile	0.84	1.81	0	1.55	1.03	1.61	0	1.90
L-Leu	0	0.1	0	0	0	0	0	0.32
L-Val	2.4	1.91	0	2.80	1.88	1.88	0	2.27
L-Phe	0	0	0	1.04	0.13	0	0	1.82
L-Trp	0.36	0.49	0	0.66	0.28	0.46	0	0.69
DL-Met	2.44	3.59	3.02	2.68	3.08	3.83	3.00	3.25
L-Thr	1.70	2.53	0	1.21	2.05	3.21	0	2.38
L-Lys-HCl	3.87	3.88	0	4.65	4.30	4.30	0	4.63
L-Tyr	0.32	0.29	0	0.90	0.18	0	0	0.68
L-Ala	0	0	0	3.00	0	0	0	2.40
L-Asp	0	0	0	3.40	0	0	0	3.30
L-Glu	0	0	0	5.10	0	0	0	5.0
Zinc-Bac.	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Coccidiostat Narasin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Minerals + vitamins	8.36	8.36	8.36	8.36	8.36	8.36	8.36	8.36
Chalk	5.0	5.0	7.0	4.0	7.3	7.3	7.5	5.5
Mono-Ca-phosphate	9.99	9.99	10.0	8.10	6.50	6.50	4.50	3.40
$NaHCO_3$	4.0	4.1	0	7.5	3.0	3.0	0	7.5

5) Diet 1 with extra threonine up to the level of diet 2. This diet was designed to test whether the difference in Thr content between diet 1 and 2 was a probable cause of differences between these diets. (1 + Thr)
6) Diet 1 with in phase 1 (0-14 days of age) less and in the subsequent phase (15-35 d) more (1 g/kg) methionine. This treatment was based on the results of *in-vivo* metabolic research. (ten Doeschate et al., 1995c) (1- + Met)
7) Diet 2 with extra glutamic acid (Glu) up to the level of Glu + Ala + Asp in diet 3 to test a possible lack of non-essential amino acids in diet 2. (2 + Glu)
8) Results of this diet are not presented because diet composition was not as intended .
9) The amino acid pattern of this diet was similar to that of diet 1 but 25 % of Glu + Asp + Ala contents were included as synthetic amino acids. To be able to do this, this diet contained less normal protein sources and more synthetic amino acids. (CC)
10) Diet 9 minus 25 % of Asp (-Asp)
11) Diet 9 minus 25 % of Ala (-Ala)
12) Diet 9 minus 25 % of Glu (-Glu)
13) Diet 9 minus 25 % of Asp + Ala + Glu (-NEA)
14) Diet 9 minus 12.5 % of Asp + Ala + Glu (-½NEA)
15) Diet 9 plus 12.5 % of Glu (+ ½NEA)
16) Diet 9 plus 25 % of Glu (+ Glu)
17) Diet 9 plus 25 % of Glu and an equimolar amount of Gly (+ NEA)
18) Diet 13 minus 25 % of Thr
19) Diet 13 plus 25 % of Thr
20) Diet 9 minus 25 % of Thr
21) Diet 9 plus 25 % of Thr
22) Diet 16 minus 25 % of Thr
23) Diet 16 plus 25 % of Thr

The composition of diet 1, 2, 3 and 9 in both respective growth phases is given in table 1. Other diets were composed by exchange of amino acids from or to diet 1, 2 or 9. The calculated ileal digestible amino acid contents of diets 1 to 7 and 9 in both growth phases are given in table 2. These were per diet calculated based on ileal amino acid digestibility coefficients, calculated from the coefficients per feedstuff (Scheele et al., 1992) combined with dietary inclusion rate, and the analyzed dietary amino acid content.

Table 2. Dry matter (DM), apparent ileal digestible amino acid and nitrogen (N) content of diets 1 to 7 and 9 (g/kg diet). Calculations based on ileal digestibility coefficients and analysed amino acid contents. A = Growth phase 1, 0-14 d of age; B = Growth phase 2, 15-35 d of age. The numbers of the diets correspond with the descriptions given in the text.

	1A	2A	3A	4A	5A	6A	7A	9A	1B	2B	3B	4B	5B	6B	7B	9B
DM	902.0	902.4	900.3	903.8	903.0	902.9	904.3	908.1	900.1	901.6	899.7	900.3	900.8	900.8	902.1	904.1
Cys	1.4	1.5	1.8	1.6	1.5	1.5	1.5	1.2	1.3	1.5	2.0	1.5	1.7	1.5	1.5	1.3
Asp	12.0	12.0	17.3	12.0	12.1	12.0	12.1	12.9	11.5	12.0	15.6	12.2	12.4	12.2	11.9	12.8
Met	5.0	6.0	5.1	4.9	4.6	4.1	5.7	5.1	4.9	5.6	5.6	5.0	5.2	5.7	5.4	5.8
Thr	7.1	8.0	6.6	7.1	79	7.0	7.8	7.0	6.6	8.1	6.3	6.9	8.1	6.8	8.1	7.2
Ser	6.8	6.8	9.1	6.9	6.6	6.7	6.7	5.5	6.5	6.7	8.7	6.7	6.8	6.7	6.7	5.3
Glu	27.8	28.1	35.1	27.9	27.0	27.1	42.3	26.0	26.3	26.9	32.6	27.2	27.3	27.1	40.1	24.4
Gly	14.9	15.1	9.8	15.1	14.3	14.7	14.7	15.8	11.5	11.5	8.6	11.6	11.7	11.6	11.9	13.2
Ala	8.9	8.9	9.6	8.9	8.7	8.9	8.8	12.8	7.9	8.0	8.5	8.2	8.2	8.1	8.1	11.4
Val	10.0	9.6	9.0	10.0	10.1	10.0	9.5	9.8	8.3	8.6	8.2	8.7	8.6	8.6	8.4	0.83
Ile	7.1	8.1	8.2	7.0	7.0	7.1	8.1	7.0	7.0	7.7	7.9	7.1	7.1	7.1	8.2	0.7
Leu	12.4	12.5	14.7	12.3	12.1	12.2	12.3	12.0	11.6	11.9	13.5	11.8	11.7	11.8	12.0	11.6
Tyr	4.7	5.1	6.4	4.9	4.6	4.7	4.7	4.6	4.6	4.7	6.9	4.8	4.7	4.7	4.6	4.4
Phe	6.8	6.8	9.1	6.9	6.6	6.6	6.7	6.5	6.4	6.5	7.6	6.6	6.6	6.5	6.6	6.7
His	6.0	3.7	4.7	4.9	5.9	5.9	3.7	5.6	5.4	3.3	4.1	4.3	5.3	5.2	3.2	5.3
Lys	10.8	10.7	10.9	11.2	11.1	11.0	10.9	10.9	9.9	10.2	10.2	10.3	10.3	9.9	10.0	10.0
Arg	11.1	11.7	14.8	12.5	11.9	12.2	12.7	11.2	9.9	10.9	11.7	9.8	10.0	9.1	10.9	10.3
Pro	10.4	10.3	11.8	10.4	10.1	10.1	10.0	9.2	8.6	9.5	10.6	9.9	9.6	9.6	10.1	9.4
N (Il. dig.)	25.5	25.2	27.9	25.2	25.4	25.2	26.6	25.8	24.7	24.9	26.9	24.3	24.8	24.8	25.9	24.3

Measurements

Growth and feed consumption were determined weekly from 0-35 days of age. Birds were weighed as a group of 15 at the beginning of a light period per room. The light schedule of consecutive rooms had a half an hour difference to give opportunity for weighing in all rooms to take place at a similar moment with regard to the lighting regime. Before the weighing at two and five weeks of age birds were withheld food for at least four hours in order to have minimal gut fill. At these ages per pen three birds were taken, euthanized with CO_2 and frozen for subsequent total body analysis. Two samples of ten day-old chicks were taken at the beginning of the experiment. These samples were, as a group (10 day-old or 3 older chicks) minced when still frozen. Subsequently maximal 1000 g of material was mixed with celite (70:30 on a fresh weight basis) to absorb and disperse body fat. Carcass samples with celite were freeze-dried. Afterwards, samples were ground to pass a 1 mm sieve.

Calculations were made of growth rate, accretion of nitrogen and energy, efficiency of deposition of nitrogen, both based on total and ileal digestible nitrogen and of efficiency of energy deposition. The last parameter was measured to find out whether dietary amino acid supply affected efficiency of energy deposition in the body.

Analysis

Before composing the diets all foodstuffs to be used were analyzed for nitrogen, fat, cell walls and amino acids. From each supply of diet and from the remaining food at the end of the experiment dry matter content was determined. From each feed a sample was analyzed, in duplo, for dry matter, nitrogen, caloric value and amino acids.

Nitrogen content was determined by a standard Kjehldahl procedure (NEN 3145). Energy contents were determined by total combustion in an adiabatic bomb calorimeter (NEN/ISO 1928). For amino acid analysis an acid and separate oxidative hydrolysis was performed. After passage through a LC-ion column (Beckmann LC 5001) amino acids were derivatised with ninhydrin. Norleucine was used as internal standard. Values for ser, thr, ile and val were corrected according to Slump (1969). Contents in carcass samples were recalculated to contents in chicks in the pen based on the relative amount of celite added and mean weight of chicks in the pen and in the samples.

Statistics

Statistical analysis was done by analysis of variance, using Genstat (Genstat 5 Committee, 1993). Room was used as the factor block. The models used are given for each experiment separately. Effects were tested for significance using an F-test. In case of a significant effect ($P<0.05$) a subsequent t-test was performed to reveal significant contrasts. Results are given as mean values and standard errors of difference (sed).

Experimental design:

Experiment 1

Differences between dietary amino acid patterns were studied in diets 1 to 7. The 'practical' diet (3) had a composition which was quite different from the six other diets which were composed of a basal mixture combined with synthetic amino acids. The model used was:

$$Y = \mu + block_i + diet_j + e_{ijk} \qquad \text{[Model 1]}$$

in which Y = response parameter; μ = mean; block = blockfactor ($i=1..3$; three rooms); diet = effect of dietary amino acid pattern ($j=1..7$); e = random error ($k=1,2$; repetitions within rooms).

Experiment 2

In the second experiment several levels of non-essential amino acids were compared at a single level of essential nitrogen. Diets 9 to 17 were used. The data from this part were analyzed in two ways:
At first with the following model:

$$Y = \mu + block_i + diet_j + e_{ij} \qquad \text{[Model 2]}$$

In which block = blockfactor ($i=1..6$; six rooms); diet = the different diets with differences in non-essential amino acid content ($j=1...9$).

Secondary the data were analyzed by linear regression in which the independent variable was the % of NEAA in the diet relative to diet 9. Model:

$$Y = \text{Constant} + \text{block}_i + b * \%\text{NEAA-N} + e \quad \text{[Model 3]}$$

in which Constant = fitted constant; block = contribution of factor block (i = 1..6); %NEAA-N = the non-essential amino acid content in the diet relative to diet 9; b = fitted slope of regression.

Experiment 3

In the third experiment the effect of non-essential N-content on threonine requirement was determined. For this purpose a 3 x 3 factorial design was used. Diets 9, 13, 16 and 18 to 23 were compared based on their NEAA and Thr content. The model used was:

$$Y = \mu + \text{block}_i + \text{NEAA}_j + \text{Thr}_k + \text{NEAA*Thr}_{jk} + e_{ijk}$$

In which block = blockfactor (i = 1..6); NEAA = level of non-essential amino acids (j = 1..3); Thr = level of threonine (k = 1..3); NEAA*Thr = interaction between NEAA level and Thr-level.

Results

Experiment 1

Performance parameters are shown in table 3. Data of growth phase 1 (0-14) and 2 (15-35 days of age) are shown separately. For the combined results over the whole growth period only the results of the statistical analyses are given. In the first growth period (0-14 d of age) several significant effects were found. Feed intake was not significantly affected by diet. Growth and N-accretion were higher for the practical diet than for all experimental diets. Within the experimental diets no significant differences were found. This resulted in a lower FCR for the birds fed the practical diet compared to all other diets. Within the experimental diets diet 2 supplemented with glutamic acid (diet 7) had a

Table 3. Performance experiment 1

	Accretion			Intake				Efficiency			
	Growth	N	GE	Food	N	GE	Dig. N	FCR	N	GE	Dig. N
Growth phase 1: 0-14 d											
1: tD	25.6^{ab}	0.591^{a}	517	32.8	1.035^{a}	1348	0.832^{ab}	1.28^{bca}	57.1	38.4^{bc}	71.1
2: Baker	25.5^{a}	0.599^{a}	510	33.0	1.036^{a}	1367	0.833^{ab}	1.29^{c}	57.8	37.3^{ab}	72.0
3: Prac.	26.9^{b}	0.659^{b}	505	33.0	1.155^{b}	1355	0.914^{c}	1.22^{a}	57.1	37.3^{ab}	72.1
4: 1-his	25.6^{ab}	0.604^{a}	513	32.9	1.027^{a}	1359	0.824^{ab}	1.28^{bc}	58.8	37.8^{abc}	73.3
5: 1 + thr	25.2^{a}	0.601^{a}	492	32.5	1.021^{a}	1340	0.822^{ab}	1.29^{bc}	58.8	36.7^{a}	73.2
6: 1-met	24.9^{a}	0.576^{a}	515	31.9	0.992^{a}	1310	0.797^{a}	1.28^{bc}	58.1	39.3^{c}	72.4
7: 2 + glu	25.1^{a}	0.596^{a}	504	31.9	1.033^{a}	1313	0.840^{b}	1.27^{b}	57.7	38.4^{bc}	71.0
F-prob.	0.003	<0.001	0.548	0.191	<0.001	0.137	<0.001	<0.001	0.430	0.001	0.418
sed	0.462	0.0108	13.3	0.566	0.0183	23.73	0.0147	0.0084	0.935	0.545	1.17
Growth phase 2: 15-35 d											
1: tD	70.8	1.97	1714	116	3.63^{a}	4870	2.87^{a}	1.64^{b}	54.3	35.2	68.7^{ab}
2: Baker	70.2	1.95	1704	114	3.60^{a}	4845	2.84^{a}	1.63^{b}	54.3	35.1	68.8^{ab}
3: CLO	71.4	2.02	1628	112	3.85^{b}	4829	3.02^{b}	1.57^{a}	52.5	33.7	66.8^{ab}
4: 1-his	70.4	1.96	1739	116	3.60^{a}	4915	2.83^{a}	1.65^{b}	54.6	35.4	69.3^{ab}
5: 1 + thr	70.2	1.97	1684	114	3.57^{a}	4828	2.82^{a}	1.62^{ab}	55.1	34.9	69.6^{ab}
6: 1 + met	70.0	2.0	1694	114	3.58^{a}	4842	2.83^{a}	1.63^{b}	55.8	35.0	70.6^{b}
7: 2 + glu	68.9	1.90	1621	112	3.62^{a}	4727	2.89^{a}	1.62^{ab}	52.8	34.3	66.9^{a}
F-prob	0.560	0.305	0.307	0.069	<0.001	0.290	<0.001	0.001	0.048	0.663	0.029
sed	1.20	0.046	55.1	1.66	0.0537	70.9	0.0426	0.0167	0.903	0.923	1.353
Statistical analysis combined results total growth period: 0-35 d											
F-prob	0.145	0.086	0.209	0.048	<0.001	0.166	<0.001	<0.001	0.028	0.480	0.016
sed	0.74	0.027	32.2	1.04	0.034	44.5	0.027	0.013	0.903	0.75	1.14

^{abc} Within a column, means with same letter are not significant different (t-test; $P<0.05$)

significant lower FCR than diet 2. N-efficiency, based on total N-content of the diets, was not significantly affected by dietary composition. The diet with less methionine (diet 6) had a higher energetic efficiency (GE %) than the diet with extra threonine. In the second period (15-35 d) no significant effects on feed intake nor on accretion were found. Nitrogen intake was significantly higher for the practical diet than for other diets. FCR of the practical diet was significantly lower than of most experimental diets. Although not significant at the 5 % level, nitrogen efficiency of the practical diet tended to be lower than that of most of the experimental diets. The efficiency of ileal digestible nitrogen of diet 6 (diet 1 (carcass-pattern, ten Doeschate et al., 1995a) with extra methionine) was significantly higher than that of diet 7 (diet 2 (Baker & Han, 1994) with extra glutamic acid). When the whole growth period was concerned, feed intake of the practical diet tended to be lower than that of the experimental diets. Accretion was not affected by dietary composition, resulting in a significant lower FCR for the practical diet compared to the experimental diets. Efficiency of ileal digestible nitrogen was lowest for diet 2 supplemented with glutamic acid and highest for diet 1 with varied methionine content. The other diets obtained intermediate results without significant effects.

Experiment 2

Performance parameters are shown in table 4. Data of growth phase 1 (0-14) and 2 (15-35 days of age) are shown separately. For the combined results over the whole growth period only the results of the statistical analysis are given. In the first growth period (0-14 d of age) feed intake of all diets was similar resulting in differences in (digestible) N-intake according to the relative N-content of the diets. Growth as well as nitrogen deposition were affected by the NEAA content of the diet. The diet with the lowest NEAA content (13) exhibited lowest growth whereas the highest growth rate was measured for the diets with extra glutamic acid. The same effect was found for nitrogen retention but for energy retention there were no significant effects. FCR was highest in the low-NEAA diets and lowest in the NEAA supplemented diets. Nitrogen efficiency, based both on total and on digestible nitrogen, was lowest in the NEAA supplemented diets and highest in low NEAA diets. Especially the diet where only aspartic acid was lowered had a positive effect on N-efficiency. In the second growth phase, there were no effects on feed intake, so nitrogen intake was

Table 4: Performance experiment 2

	Accretion			Intake				Efficiency			
	Growth	N	GE	Food	N	GE	Dig. N	FCR	N	GE	Dig. N
Growth phase 1: 0-14 d											
9: KK	24.9^{bc}	.592^{bc}	491	31.7	.995^{b}	1267	0.807^{b}	1.27^{b}	59.6^{bc}	38.8	73.5^{cd}
10: -asp	24.6^{abc}	.581^{bc}	489	31.3	.961^{ab}	1255	0.776^{ab}	1.27^{b}	60.5^{c}	39.0	74.8^{d}
11: -ala	24.9^{bc}	.581^{bc}	495	31.6	.999^{b}	1282	0.807^{b}	1.27^{b}	58.1^{abc}	38.6	72.0^{bcd}
12: -glu	24.4^{ab}	.576^{bc}	485	31.2	.993^{b}	1269	0.802^{b}	1.28^{bc}	58.0^{ab}	38.2	71.8^{abc}
13: -NEA	23.6^{a}	.545^{a}	470	30.6	.940^{a}	1244	0.753^{a}	1.30^{c}	58.0^{ab}	37.8	72.4^{bcd}
14: -½NEA	24.4^{ab}	.563^{ab}	480	31.1	.981^{ab}	1265	0.791^{b}	1.28^{b}	57.5^{ab}	38.0	71.3^{abc}
15: + ½glu	25.5^{c}	.604^{c}	493	31.8	1.05^{c}	1302	0.858^{c}	1.25^{a}	57.3^{ab}	37.9	70.4^{ab}
16: +glu	25.3^{bc}	.601^{c}	495	31.5	1.05^{c}	1288	0.859^{c}	1.25^{a}	57.2^{ab}	38.5	70.0^{ab}
17: +NEA	25.3^{bc}	.594^{bc}	480	31.1	1.05^{c}	1266	0.861^{c}	1.23^{a}	56.6^{a}	37.9	69.0^{a}
F-prob.	0.026	.006	0.523	0.750	<.001	0.559	<.001	<.001	0.016	0.595	0.002
sed	0.512	0.015	12.8	0.646	0.021	26.7	0.017	0.009	1.04	0.672	1.28
Growth phase 2: 15-35 d											
9: KK	71.4	1.98^{bcd}	1740	119	3.58^{cd}	4941	2.90^{cd}	1.67^{abcd}	55.3	35.2	68.4^{bc}
10: -asp	68.6	1.88^{ab}	1691	115	3.50^{bc}	4765	2.82^{bc}	1.68^{bcd}	53.7	35.5	66.7^{ab}
11: -ala	69.1	1.89^{abc}	1725	116	3.48^{abc}	4798	2.80^{bc}	1.68^{bcd}	54.4	36.0	67.5^{b}
12: -glu	69.2	1.89^{abc}	1683	116	3.48^{abc}	4811	2.80^{bc}	1.68^{cd}	54.4	35.0	67.6^{b}
13: -NEA	67.6	1.87^{ab}	1679	114	3.33^{a}	4718	2.67^{a}	1.69^{d}	56.2	35.6	70.3^{c}
14: -½NEA	67.9	1.86^{a}	1708	115	3.42^{ab}	4774	2.75^{ab}	1.69^{d}	54.2	35.8	67.5^{b}
15: + ½glu	70.8	1.97^{bcd}	1740	117	3.59^{cd}	4837	2.92^{cd}	1.65^{abc}	54.9	36.0	67.7^{b}
16: +glu	72.1	2.02^{d}	1760	118	3.69^{de}	4899	3.01^{de}	1.63^{a}	54.6	35.9	67.0^{ab}
17: +NEA	71.4	2.00^{cd}	1662	117	3.78^{e}	4869	3.09^{e}	1.64^{ab}	52.9	34.1	64.7^{a}
F-prob	0.06	0.043	0.697	0.622	<.001	0.523	<.001	0.021	0.145	0.388	0.019
sed	1.62	0.058	56.9	2.52	0.077	105	0.062	0.019	1.03	0.81	1.27
Statistical analysis combined results total growth period: 0-35 d											
F-prob	0.026	0.020	0.583	0.604	<.001	0.522	<.001	0.001	0.115	0.337	0.007
sed	1.07	0.038	35.4	1.65	0.051	68.5	0.041	0.015	0.69	0.69	1.10

^{abc} Within a column, means with same letter are not significant different (t-test; $P<0.05$)

affected according to nitrogen content of the diets. Nitrogen retention was highest for NEAA supplemented diets and lowest for diets with lowered NEAA contents. FCR was highest for the low NEAA-diets and lowest for the supplemented diets. Efficiency of ileal digestible nitrogen was highest for the low NEAA diet and lowest for the NEAA supplemented diet. Over the whole growth period (0-35 d), effects were similar to the effects observed in both separate periods. There was a significant effect of NEAA content on nitrogen intake, growth, nitrogen retention, FCR and efficiency of ileal digestible nitrogen. High NEAA content improved growth, nitrogen retention and FCR. Efficiency of nitrogen retention however was negatively affected by high NEAA content. In figure 1 the linear regressions of body accretion, FCR and N-efficiency on NEAA content (relative to NEAA content of diet 9) are shown. From this figure it is clear that N-efficiency is affected in a positive sense by lower dietary NEAA level whereas growth rate and FCR are affected in a negative sense.

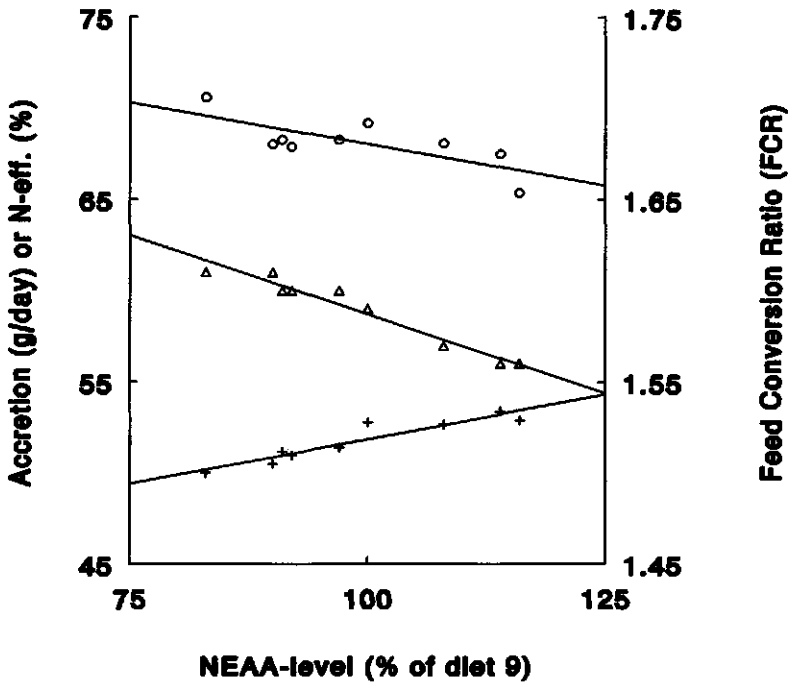

Figure 1. Effect of dietary NEAA-level (relative to that in the amino acid pattern of body accretion) on accretion, FCR and N-efficiency of ileal digestible N over 0-35 days of age.
+ = Accretion; Δ = FCR; O = N-efficiency of ileal digestible N.

Table 5. Performance experiment 3

Dietary level		Accretion			Intake				Efficiency			
NEAA thr		Growth	N	GE	Food	N	GE	Dig. N	FCR	N	GE	Dig. N
		Growth phase 1: 0-14 d										
Low	L	18.2^{b}	.424^{b}	365^{b}	25.5^{b}	.778^{b}	1025^{b}	.622^{b}	1.40^{e}	54.4^{bc}	35.5^{a}	68.0^{b}
	M	23.6^{c}	.545^{c}	470^{c}	30.6^{c}	.940^{c}	1244^{c}	.753^{c}	1.30^{d}	58.0^{d}	37.8^{b}	72.4^{cd}
	H	24.0^{cd}	.557^{cd}	484^{c}	30.8^{c}	.954^{cd}	1252^{c}	.767^{c}	1.28^{cd}	58.4^{de}	38.6^{b}	72.7^{d}
Med.	L	17.5^{b}	.403^{b}	344^{b}	24.4^{b}	.762^{b}	981^{b}	.617^{b}	1.39^{e}	52.9^{ab}	35.1^{a}	65.3^{a}
	M	24.9^{de}	.592^{e}	491^{c}	31.7^{c}	.995^{d}	1267^{c}	.807^{d}	1.27^{bc}	59.6^{e}	38.8^{b}	73.5^{d}
	H	25.0^{de}	.582de	483^{c}	31.4^{c}	.993^{d}	1273^{c}	.806^{d}	1.26^{ab}	58.6^{de}	38.0^{b}	72.2^{cd}
High	L	15.9^{a}	.369^{a}	308^{a}	22.0^{a}	.713^{a}	878^{a}	.583^{a}	1.38^{e}	51.8^{a}	35.1^{a}	63.4^{a}
	M	25.3^{e}	.601^{e}	495^{c}	31.5^{c}	1.05^{e}	1288^{c}	.859^{e}	1.25^{a}	57.2^{d}	38.5^{b}	70.0^{bc}
	H	25.6^{e}	.597^{e}	494^{c}	31.8^{c}	1.06^{e}	1287^{c}	.865^{e}	1.24^{a}	56.6^{cd}	38.4^{b}	69.0^{b}
F-prob NEAA		0.231	0.079	<0.001	0.118	<0.001	0.233	<0.001	<0.001	0.001	0.987	<.001
sed		0.278	0.008	8.01	0.363	0.012	15.0	0.009	0.006	0.514	0.439	.635
F-prob thr		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<.001
sed		0.278	0.008	8.01	0.363	0.012	15.0	0.009	0.006	0.514	0.439	.635
F-prob		<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	0.167	0.195	0.547	0.207
sed		0.481	0.014	13.9	0.628	0.020	26.0	0.016	0.010	0.890	0.760	1.10

Table 5: Performance experiment 3, continued

Dietary level		Accretion			Intake				Efficiency			
NEAA	thr	Growth	N	GE	Food	N	GE	Dig. N	FCR	N	GE	Dig. N
Growth phase 2: 15-35 d												
Low	L	62.9^{a}	1.68^{a}	1578^{abc}	106^{a}	3.05^{a}	4388^{a}	2.43^{a}	1.68^{bc}	55.0^{de}	36.0	69.0^{ef}
	M	67.6^{cd}	1.87^{cd}	1679^{cde}	114^{bc}	3.33^{b}	4718^{cd}	2.67^{bc}	1.69^{bc}	56.2^{e}	35.6	70.3^{f}
	H	66.1^{bc}	1.81^{bc}	1637^{bcd}	113^{b}	3.30^{b}	4684^{bc}	2.64^{b}	1.71^{c}	54.7^{de}	34.9	68.3^{def}
Med.	L	63.4^{ab}	1.71^{ab}	1559^{ab}	108^{a}	3.27^{b}	4498^{ab}	2.64^{b}	1.71^{c}	52.1^{bc}	34.7	64.6^{bc}
	M	71.4^{e}	1.98^{de}	1740de	119^{d}	3.58^{de}	4941^{e}	2.90^{e}	1.67^{abc}	55.3^{de}	35.2	68.4^{def}
	H	70.1^{de}	1.89^{cd}	1724de	116^{bcd}	3.52^{cd}	4810^{cde}	2.85^{de}	1.65^{ab}	53.7^{cd}	35.8	66.4^{cd}
High	L	63.2^{ab}	1.67^{a}	1487^{a}	105^{a}	3.38^{bc}	4334^{a}	2.75^{cd}	1.66^{ab}	49.4^{a}	34.3	60.7^{a}
	M	72.1^{e}	2.02^{e}	1759^{e}	118^{e}	3.69^{ef}	4899de	3.01^{f}	1.63^{a}	54.6^{de}	35.9	67.0^{cde}
	H	69.2^{de}	1.94^{de}	1659^{bcde}	116^{bcd}	3.78^{f}	4828^{cde}	3.09^{f}	1.68^{bc}	51.4^{ab}	34.4	62.9^{ab}
F-prob NEAA		0.003	0.008	0.290	0.024	<0.001	0.021	<0.001	0.024	<0.001	0.344	<0.001
sed		0.84	0.030	29.9	1.27	0.038	52.9	0.031	0.012	0.571	0.422	0.709
F-prob thr		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.260	<.001	0.322	<0.001
sed		0.84	0.030	29.9	1.27	0.038	52.9	0.031	0.012	0.571	0.422	0.709
F-prob		0.247	0.252	0.184	0.561	0.380	0.362	0.356	0.036	0.075	0.101	0.079
sed		1.45	0.051	51.8	2.20	0.066	91.7	0.053	0.020	0.990	0.730	1.23
Statistical analysis combined results total growth period: 0-35 d												
F-prob		0.002	0.003	0.276	0.038	<0.001	0.041	<0.001	0.004	<0.001	0.308	<.001
sed		0.539	0.018	18.5	0.839	0.025	35.0	0.021	0.009	0.484	0.349	.600
F-prob		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.028	<.001
sed		0.539	0.018	18.5	0.839	0.025	35.0	0.021	0.009	0.484	0.349	.600
F-prob		0.032	0.032	0.059	0.186	0.128	0.100	0.106	0.021	0.036	0.080	0.039
sed		0.934	0.031	32.1	1.45	0.044	60.6	0.036	0.016	0.838	0.605	1.04

[abc] Within a column, means with same letter are not significant different (t-test; P<0.05)

Experiment 3

Performance parameters are shown in table 5. Data of growth phase 1 (0-14) and 2 (15-35 days of age) are shown separately while for the combined results over the whole growth period only the results of the statistical analysis are given.

In the first growth phase (0-14 d), interaction between Thr and NEAA level was found to be statistically significant for all intake and retention parameters. The combination of low Thr and high NEAA content generally resulted in the most severe effect. For all parameters mentioned in table 5 the effect of Thr level was significant whereas the effect of NEAA content was significant for energy retention, N-intake, digestible N-intake, FCR and efficiency of N retention. In general, low Thr and low NEAA levels caused results significant different from medium and high levels whereas medium and high levels displayed smaller or no differences.

In the second growth phase (15-35 d), interaction between Thr and NEAA was not significant except for FCR (F-probability = 0.036). This interaction was caused by lack of effect of Thr level in low NEAA diets whereas in medium NEAA diets low Thr level was worse than high Thr level whereas at high NEAA level the medium Thr level gave a better FCR than the high Thr-level. The effect of Thr-level was significant for all retention and intake parameters and for efficiency of nitrogen retention. Low Thr-level clearly resulted in reduced feed intake, rate of gain, increased FCR and lower N-efficiency. The NEAA content of the diet had a significant effect on growth, N-retention, intake parameters, FCR and efficiency of nitrogen retention.

When the entire growth period was considered the interaction between Thr-level and NEAA content was statistically significant ($P<0.05$) for growth, N-retention, FCR and efficiency of N-retention. For growth and N-retention this was a result of a larger effect of low Thr-level at the high NEAA content than at the low NEAA content. For FCR and efficiency of N-retention Thr-level had no effect at low NEAA content but resulted in worse FCR or efficiency in diets with medium or high NEAA content.

Discussion

In the following discussion we will try to answer the three main questions raised in the introduction. Firstly, can nitrogen efficiency be improved by feeding diets of which the amino acid pattern was based on requirements values or on amino acid pattern of body accretion compared to a practical diet ? Can nitrogen efficiency be positively affected by changes in these patterns based on physiological considerations ? These questions will be answered based on the results of *experiment 1.* Secondly, in *experiment 2* was tested whether re-utilisation of non-essential amino acids formed in the degradation process of essential amino acids for protein synthesis could be stimulated through lower dietary NEAA levels and thus result in higher N-efficiency. Thirdly, in *experiment 3* the hypothesis was tested that high dietary NEAA level would, through utilisation of threonine as glycine source, result in higher threonine requirement than low NEAA level.

Experiment 1

Based on the assumption that in a practical diet requirements would not be used as strictly as in the experimental diets it was expected that the practical diet would result in lower N-efficiency than the experimental diets. The practical diet indeed resulted in lower nitrogen efficiency than the experimental diets. However, the magnitude of the difference was not large. Also the absolute level of nitrogen efficiency (more than 65 % of ileal digestible nitrogen is used for body accretion) was very high. This implies that the practical diet used in this experiment was very good adapted to requirements. It should be realized that in practice males and females are grown together which will result in much lower overall efficiency (ten Doeschate et al., 1995b). The difference in N-efficiency between practical and experimental diets shows that the excess of some amino acids in the practical diet relative to requirement resulted in lower N-efficiency.

It is shown in a previous paper (ten Doeschate et al., 1995a) that the amino acid pattern as advised by Baker and Han (1994) does not differ very much from the pattern found in body accretion . The technical performance attained by the broiler chicks in the present investigation was similar when fed either the Baker or the ten Doeschate pattern. The main differences between both patterns, namely Thr and His content were investigated by diet 4 and 5. It was shown that these changes did not result in significant lower performance.

From this it can be concluded that: 1) The Thr level as used in diet 1 was sufficient, and 2) It is not necessary to reflect the high His level as found in body accretion (ten Doeschate et al., 1995a) in dietary composition. It is shown that addition of glutamic acid had no positive effect which implies that glutamic acid content of diet 2 (and also diet 1) is already high enough and there is thus no shortage of non-essential amino-N. The variation in dietary methionine in both phases (i.e. relative to body accretion lower in growth phase 1 and higher in growth phase 2) resulted in better performance. This suggests that dietary methionine content could be lower than that found in amino acid pattern of body accretion in the first growth phase but should be higher in the second growth phase. This latter finding is in agreement with several investigation where requirements of methionine were determined (eg Schutte & Pack, 1993). The fact that methionine content may be less critical during the first part of the growth phase however is not previously reported.

The main conclusion from experiment 1 is that changes in amino acid pattern within experimental diets resulted in small effects, indicative of a not very high sensitivity of amino acid metabolism to relative small variations in dietary amino acid pattern. This will only be valid as long as non-limiting amino acids are varied.

Experiment 2

The experiment was designed to test the consequences of the hypothesis that the non-essential amino acids (NEAA, glu + ala + asp) formed from obligatory degradation of essential amino acids can, by lowering dietary NEAA level, be incorporated into body protein. It was shown that efficiency of nitrogen accretion decreases with higher dietary NEAA content. This can be considered as evidence for re-utilisation of nitrogen in non-essential amino acids. However, the percentage depression in growth of birds given low NEAA diets was more than the percentage increase in nitrogen efficiency. This implies that, when an economic evaluation is made, the very low NEAA diets will probably not be useful. However, it is demonstrated that the hypothesis is correct. Furthermore, in most common vegetable foodstuffs used in poultry diets, NEAA contents relative to essential amino acids are higher than in the amino acid pattern of body accretion. This means that feed industry will not be able to reduce NEAA contents of the feeds to the low levels used in this study. But, since it is shown

that higher levels of NEAA result in lower N-efficiency without better growth it would be advisable to strive for lower NEAA values in diet formulation.

A more physiological discussion of effects of low NEAA diets on growth and efficiency requires more knowledge on the processes of amino acid catabolism and uric acid production. Basically one would expect that NEAA produced from essential amino acid catabolism could be used for nett protein synthesis. Apparently, judging from the growth data on the low NEAA diets, there is a shortage in NEAA at the protein synthesis level. However, there is still uric acid production (30 % of absorbed nitrogen is excreted as uric acid). In these diets it is thus shown that a high efficiency of non-essential amino acids does not mean that these amino acids are in total limiting for growth and thus preventing a high efficiency for essential amino acids as suggested by Scheele (1972) in studies with rats. The excretion of uric acid means that at the site where uric acid is produced there is still an excess of NEAA whereas at protein synthesis sites NEAA levels are lower than optimal for maximal growth rate. Probably the NEAA produced from amino acids catabolized in the liver is mainly channelled directly to uric acid synthesis instead to the sites of nett protein synthesis. Subsequent more metabolism oriented studies should clarify this point.

Experiment 3

The hypothesis on which this experiment was based was that excess of NEAA would result in extra uric acid production. Moreover the consequence would be an increased threonine requirement. Threonine requirement would be higher because threonine is a precursor for glycine synthesis. In the synthesis of glycine from threonine also a an active formate unit is produced (Stryer, 1988), which can be utilized for uric acid synthesis. In the 3x3 factorial design chosen the hypothesis would be confirmed if significant interaction between threonine and NEAA levels were found. In the first growth phase (0-14d) and when the whole period was considered (0-35 d), several interactions were noted. However, in the second growth phase interaction effects, except for FCR, were not significant. Of course, phases were not independent since groups of chicks were kept on the same type of diet in both phases. The finding that in the second growth phase interaction was not significant can either be explained by lower sensitivity in older chicks or by adaptation during growth. However, the fact that in the early growth phase interaction between NEAA and Thr was as

expected indicates that the original hypothesis was correct. This means that in diets with high nitrogen excess threonine requirement will be higher than in well adjusted diets such as a diet based on the amino acid pattern of body accretion. In other words, threonine requirement is, above the amount required for body accretion, dependent on nitrogen excess of the diet and can thus not be considered a fixed value.

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Chapter 7

MEAT PRODUCTION OF MALE BROILERS FED DIETS DIFFERING IN AMINO ACID PATTERN, NON-ESSENTIAL NITROGEN AND THREONINE CONTENT.

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Summary

Meat production of broilers, both absolute and relative to live weight could be affect by dietary amino acid pattern, dietary non-essential nitrogen supply and threonine content.In three sub-experiments these influences were studied.

In the first experiment a comparison is made between a practical diet and experimental diets based on either literature values or the amino acid pattern of body accretion plus some adaptations of these patterns. It was shown that absolute meat production was only slightly affected by dietary amino acid pattern while for relative yields several significant but minor differences were found. Breast meat percentage was highest in birds fed a diet with extra Met and lowest in birds fed a diet with extra Glu. It is concluded that within three proposed dietary amino acid patterns variation in dietary amino acid pattern had only minor influence on slaughter efficiency.

In the second experiment was studied how a decrease in dietary non-essential amino acid level (to promote utilisation of non-essential amino acids formed in the obligatory break-down of essential amino acids for nett protein synthesis) affects meat production and yields. Hereto male broiler chicks fed diets with varying levels of glutamic acid + aspartic acid + alanine (NEAA) were slaughtered and yields were determined. It was shown that variation in dietary NEAA level had no effect on yields of body parts as percentage of live weight. Total production was related to live weight and was decreased with decreasing dietary NEAA level.

In the third experiment a distinct interaction between dietary Thr and NEAA level on yields was observed. This supports the hypothesis that the requirement of threonine is determined not only by the requirement for body accretion but also by a requirement for glycine production to excrete excess nitrogen as uric acid.

Introduction

Improvement of nitrogen efficiency in animal production is of major concern in order to reduce environmental pollution with for instance ammonia (ten Doeschate et al, 1995). Nitrogen efficiency per se does not need to be the best indicator for performance. The main parameter of concern is the production of edible meat per unit of consumed nitrogen. This is to prevent that a high nitrogen efficiency is attained by a relative large nitrogen deposition in unedible parts (feathers, gut). There are some indications that dietary amino acid composition will affect relative weights of body components or slaughter efficiencies. (e.g. Moran et al., 1992). High dietary glutamic acid (or glutamine) levels were found to increase proportion of breast meat (Moran et al., 1992). We determined nitrogen efficiency by a comparative slaughter technique (ten Doeschate et al., 1995) . In three experiments in total 22 diets were used. The first experiment was set up to compare diets of which the amino acid composition was based either on literature values, practical standards, or the amino acid pattern of body accretion. Within this experiment also diets with intermediate amino acid levels were compared. In the second experiment the effect of dietary NEAA level on nitrogen efficiency was studied. This was based on the hypothesis that a lower dietary NEAA level would result in more re-utilisation of N from degraded amino acids for protein synthesis. In the third experiment the hypothesis was tested that increase of dietary N-excess would increase Thr requirement if dietary Gly and Ser level were not increased. It was shown that N-efficiency is influenced by dietary amino acid pattern and by dietary NEAA content. Moreover, the hypothesis that dietary N-excess affects Thr requirement was supported by the results of the third experiment. In the results presented here the absolute and relative yield of edible meat in the birds of these three experiments will be discussed.

Materials and methods

Birds and husbandry

Three experiments were performed with a total of 2070 male birds from a commercial broiler hybrid (HYBRO, Euribrid, Boxmeer, the Netherlands). The birds

were raised in litter floor pens (75 * 97 cm). Per environmentally controlled room 16 pens were available. Birds from experiment 1 (8 diets, *Exp. design*) were placed in three rooms with per diet two replicate pens per room. Birds from experiment 2 and 3 (15 diets, *Exp. design*) were placed in six rooms with one replicate pen per diet per room. Birds were fed ad libitum. For the first 3 d there was continuous light. Starting from three days of age, to prevent leg disorders and to stimulate a regular feed intake, the birds were kept under a lighting regime of 1 h light alternating with 2 h of darkness. Day-old chicks were vaccinated against Newcastle Disease (NCD) and Infectious Bronchitis. At 17 days of age birds were vaccinated against bursal (Gumboro) disease and at 21 days of age revaccinated against NCD. Two diets were fed subsequently from 0-14 and 15-35 days of age. At 35 days of age (exp. 1) or 36 days of age (exp. 2 and 3) birds were slaughtered to determine slaughter efficiency and yield.

Slaughter efficiency

Slaughter efficiency was determined according to the method described by Uijttenboogaard et al (1982) which is adopted as the official method of the World Poultry Science Association (WPSA). In this method birds or carcasses are weighed at several stages during the process of dissection and accordingly several yields can be calculated. Slaughter efficiency efficiency of dissection is defined as the amount of product obtained relative to some reference weight. All efficiencies, except for oven-ready, are expressed relative to live weight. These are: 1) Slaughter efficiency = carcass after removal of feathers, head and legs divided by live weight; 2) oven-ready efficiency = griller including neck, without abdominal fat pad, divided by slaughtered weight; 3) edible organs (heart + liver + gizzard); 4) abdominal fat pad; 5) griller; 6) wings; 7) breast meat; 8) skin and fat; 9) remaining carcass; 10) thighs; 11) drumsticks; 12) neck skin; 13) neck; 14) total fat offal (abdominal fat pad + skin and fat + neck skin)

Experimental design

In total 22 diets were used in the three experiments. The experiments were performed simultaneously but analysis was made within each experiment. Some experimental groups were used in the analysis of both experiment 2 and 3. Dietary composition was described in detail previously (ten Doeschate et al, 1995). Diets

were composed to vary in some amino acids without variation in feedstuffs in order to test the effects of amino acid supply as such. Two growth phases were used, 0-14 and 15-35 days of age. Diets for each growth phase were composed according to a similar principle. In total there were 23 diets. Diets 1,2 and 4-8 were based on a basal mixture supplemented with amino acids. Also diets 9-23 were composed in a similar way. Per growth phase, all diets were isocaloric and had a similar apparent ileal digestible lysine content. The only difference between diets was thus the amino acid content relative to lysine. The individual diets are described below with abbreviations in brackets:

1) The amino acid pattern was based on the amino acid pattern of body accretion in the subsequent growth phases as determined by ten Doeschate et al, 1995b) (tD)
2) The amino acid pattern was based on Baker & Han, 1993 (Baker)
3) This diet was composed to constitute a diet similar to practical diets in use in the Netherlands. (Prac)
4) Diet 1 with a lower level of histidine (His); intermediate between diet 1 and 2. This diet was designed to test whether the high level of His found in body accretion (ten Doeschate et al., 1995a) was necessary for optimal performance. (1-His)
5) Diet 1 with extra threonine up to the level of diet 2. This diet was designed to test whether the difference in Thr content between diet 1 and 2 was a probable cause of differences between these diets. (1 + Thr)
6) Diet 1 with in phase 1 (0-14 days of age) less and in the subsequent phase (15-35 d) more (1 g/kg) methionine. This treatment was based on the results of *in-vivo* metabolic research. (ten Doeschate et al., 1995c) (1- + Met)
7) Diet 2 with extra glutamic acid (Glu) up to the level of Glu + Ala + Asp in diet 3 to test a possible lack of non-essential amino acids in diet 2. (2 + Glu)
8) Results of this diet are not presented because diet composition was not as intended.
9) The amino acid pattern of this diet was similar to that of diet 1 but 25 % of Glu + Asp + Ala contents were included as synthetic amino acids. To be able to do this, this diet contained less normal protein sources and more synthetic amino acids. (CC)
10) Diet 9 minus 25 % of Asp (-Asp)
11) Diet 9 minus 25 % of Ala (-Ala)
12) Diet 9 minus 25 % of Glu (-Glu)
13) Diet 9 minus 25 % of Asp + Ala + Glu (-NEA)
14) Diet 9 minus 12.5 % of Asp + Ala + Glu (-½NEA)

15) Diet 9 plus 12.5 % of Glu (+ ½ Glu)
16) Diet 9 plus 25 % of Glu (+ Glu)
17) Diet 9 plus 25 % of Glu and an equimolar amount of Gly (+ NEA)
18) Diet 13 minus 25 % of Thr
19) Diet 13 plus 25 % of Thr
20) Diet 9 minus 25 % of Thr
21) Diet 9 plus 25 % of Thr
22) Diet 16 minus 25 % of Thr
23) Diet 16 plus 25 % of Thr

The total experiment was divided into three sub-experiments where in each sub-experiment one general hypothesis was tested. The design of these three experimental parts, further referred to as experimetnts 1, 2 and 3, is given below, combined with the statistical models used in each experiment.

Experiment 1

Differences between dietary amino acid patterns were studied in diets 1 to 8. The 'practical' diet (3) had a composition which was quite different from the seven other diets which were composed of a basal mixture combined with synthetic amino acids. The model used was:

$$Y = \mu + \text{block} + \text{diet} + e \qquad \text{[Model 1]}$$

in which Y = response parameter; μ = mean; block = room was used as factor block at three levels; diet = effect of dietary amino acid pattern; e = random error.

Experiment 2

In the second experiment several levels of non-essential nitrogen were compared at a single level of essential nitrogen. Diets 9 to 17 were used. The data from this part were analyzed in two ways: At first with the following model:

$$Y = \mu + \text{block} + \text{diet} + e \qquad \text{[Model 2]}$$

In which diet stands for the different diets with differences in non-essential amino acid content and block is room used as the factor block at six levels. Secondary the data were analyzed by linear regression in which the independent variable was the % of NEAA in the diet relative to diet 9.

Model:

$$Y = \mu + \text{block} + \%\text{NEAA-N} + e \qquad \text{[Model 3]}$$

in which %NEAA-N stands for the non-essential nitrogen content in the diet relative to diet 9.

Experiment 3

In the third experiment the effect of non-essential N-content on threonine requirement was determined. For this purpose a 3 x 3 factorial design was used. Diets 9, 13, 16 and 18 to 23 were compared based on their NEAA and Thr content. The model used was:

$$Y = \mu + \text{block} + \text{NEAA} + \text{Thr} + \text{NEAA} * \text{Thr} + e \qquad \text{[Model 4]}$$

In which NEAA denotes one of three levels of non-essential amino acids; Thr denotes one of three levels of threonine while NEAA*Thr denotes the interaction between NEAA level and Thr-level.

Statistical analysis

Yield of portions was expressed relative to live weight immediate before slaughter. Data were analysed with Genstat (version 5.3, Genstat 5 Committee, 1993). Normal and constant variation of data was checked graphically by normal plots and plots of residuals against fitted values. Paired t-tests ($t<0.001$) were performed with the procedure RPAIR if the results of ANOVA analysis revealed significant ($P<0.005$) treatment effects according to the respective models described above.

Table 1. Slaughter weights experiment 1

Portion:	Sla.	Oven	Org	Fat pad	Griller	Wings	Breast	Skinfat	Carcas	Thighs	Drums	Ne-sk	Neck	Tfat
1: tD	1605	1298	84.2	43.1^{abc}	1291	155	295	83.7	256	249	187	20.2	39.8^{ab}	147
2: Baker	1611	1304	83.3	46.1^{abc}	1297	154	287	79.1	259	256	192	21.9	42.4^{b}	147
3: Prac.	1579	1283	82.9	36.0^{a}	1276	154	286	76.3	258	248	189	20.1	41.5^{b}	132
4: 1-his	1564	1266	79.8	47.4^{bc}	1259	153	284	80.2	252	246	181	19.9	38.0^{a}	147
5: 1+thr	1600	1292	79.4	49.5^{c}	1285	155	289	81.8	254	257	187	20.1	38.1^{a}	151
6: 1-met	1545	1255	80.1	39.1^{ab}	1248	149	291	73.4	250	239	180	21.2	40.5^{ab}	134
7: 2+glu	1543	1246	82.8	41.3^{abc}	1239	151	266	74.3	254	250	180	20.5	41.1^{ab}	136
F-prob.	0.328	0.340	0.514	0.007	0.334	0.494	0.121	0.190	0.696	0.069	0.051	0.813	<.001	0.060
sed	36.3	29.0	2.9	3.6	28.9	3.5	9.9	4.4	5.8	5.8	4.4	1.5	1.0	7.3

^{abc} Within a column, means with same letter are not significant different (t-test; $P<0.01$)

Table 2. Slaughter efficiencies experiment 1

Portion:	Sla.	Oven	Org	Fat pad	Griller	Wings	Breast	Skinfat	Carcas	Thighs	Drums	Ne-sk	Neck	Tfat
1: tD	85.5	83.6	4.5	2.3^{ab}	68.7	8.3	15.7^{bc}	4.4	13.6^{ab}	13.2^{a}	10.0	1.1	2.1^{abc}	7.8^{ab}
2: Baker	85.5	83.7	4.4	2.4^{ab}	68.9	8.2	15.3^{ab}	4.2	13.8^{abc}	13.6^{ab}	10.2	1.2	2.3^{bc}	7.8^{ab}
3: Prac.	85.2	84.0	4.5	1.9^{a}	68.9	8.3	15.4^{abc}	4.1	14.0^{bc}	13.4^{ab}	10.2	1.1	2.2^{bc}	7.1^{a}
4: 1-his	85.3	83.7	4.3	2.6^{b}	68.6	8.3	15.5^{abc}	4.4	13.8^{abc}	13.4^{ab}	9.9	1.1	2.1^{ab}	8.1^{b}
5: 1+thr	85.4	83.6	4.3	2.7^{b}	68.6	8.3	15.4^{abc}	4.4	13.5^{a}	13.7^{ab}	10.0	1.1	2.0^{a}	8.1^{b}
6: 1-met	85.4	84.0	4.4	2.1^{ab}	69.0	8.3	16.1^{c}	4.1	13.8^{abc}	13.2^{a}	10.0	1.2	2.3^{c}	7.4^{ab}
7: 2+glu	85.3	83.6	4.6	2.3^{ab}	68.5	8.4	14.7^{a}	4.1	14.0^{c}	13.8^{b}	10.0	1.2	2.3^{c}	7.5^{ab}
F-prob.	0.982	0.714	0.163	0.006	0.672	0.635	0.009	0.178	0.009	0.015	0.526	0.664	0.001	0.030
sed	0.336	0.341	0.121	0.186	0.321	0.098	0.329	0.178	0.134	0.179	0.184	0.075	0.064	0.305

^{abc} Within a column, means with same letter are not significant different (t-test; $P<0.01$)

Legend of portions: Sla. = Slaughter weight, Oven = Oven ready weight, Org = Edible organs (liver, heart, gizzard), Fat pad = abdominal fat pad, Griller = Chilled carcass, Wings = Wings, Breast = Breast meat without skin, Skinfat = Skin plus skinfat, Carcass = remaining carcass after cutting other portions, Thighs = Thighs, Drums = Drums, Ne-sk = Neck skin, Neck = Neck, Tfat = Fat pad + Skinfat + Ne-sk; efficiencies all, except oven, relative to live weight; oven is relative to slaughter weight.

Results

Experiment 1: Variation in amino acid pattern

Absolute slaughter weights (table 1) revealed only significant differences for the abdominal fat pad and for weight of the neck. Abdominal fat pad was lowest in birds fed the practical diet (3) and heaviest in birds fed a diet with extra threonine (5). The neck was lighter in diets with extra threonine or reduced histidine (5 and 4) than in the practical diet and the diet according to Baker & Han (1993) (2 and 3). Differences in more valuable parts were not significant, probably due to high variation caused by differences in body weight.

When relative yields (percentages) are considered (table 2), it is shown that slaughter efficiency, griller, drums, wings, giblets, skin + fat were not affected by dietary amino acid variation. Variation in dietary amino acid pattern affected abdominal fat pad, total fat, breast meat, thighs, neck and carcass. Breast meat percentage was lowest in birds fed diet 7 (2 + Glu) and highest in diet 6 (tD -+ Met). The other diets all resulted in a similar breast meat percentage of ± 15.5 %. Thighs were relative smallest in diets 1 (tD) and 6 (1 -+ Met) and biggest in birds fed diet 7 (2 + Glu). The remaining carcass was largest in birds fed the practical diet (3) or diet 7 (2 + Glu) and smallest in birds fed diet 5 (1 + Thr). The same observation holds for the percentage of neck, which varies from 2.0 to 2.3 percent of live weight. Abdominal fat pad was lowest in broilers fed the practical diet whereas it was highest in diets with lower His or higher Thr content. Total fat (which encompasses abdominal fat pad, skin + fat and skin of the neck) reacts similar to abdominal fat pad. Generally, it can be stated that differences between diets were not found for the three diets differing in amino acid pattern but only for diets with additional or decreased amino acids.

Experiment 2: Variation in NEAA content

In table 3 the absolute slaughter yields of the nine diets differing in NEAA content are shown. From this table it is clear that nearly all absolute weights of portions are affected by dietary NEAA content. Only the edible organs, skin + fat, neck skin, neck and total fatty tissue were not affected. For all valuable parts, the low NEAA diet (13) produced lower weights than one or all of the NEAA supplemented diets. For the abdominal fat pad the opposite result was found.

Table 3. Slaughter weights experiment 2

Portion:	Sla.	Oven	Org	Fat pad	Griller	Wings	Breast	Skinfat	Carcas	Thighs	Drums	Ne-sk	Neck	Tfat
9: CC	1605^{b}	1318^{b}	80.6	44.1^{ab}	1312^{b}	155^{b}	304^{b}	85.3	256^{b}	256^{b}	188^{b}	20.1	43.1	150
10: -asp	1556^{ab}	1279^{b}	80.4	43.3^{ab}	1272^{b}	151^{ab}	296^{ab}	80.4	247^{ab}	245^{ab}	184^{b}	22.5	43.1	146
11: -ala	1582^{b}	1298^{b}	81.9	43.5^{ab}	1292^{b}	152^{b}	296^{ab}	80.7	253^{b}	253^{b}	189^{b}	21.0	43.5	145
12: -glu	1556^{ab}	1268^{ab}	81.6	48.9^{b}	1262^{ab}	148^{ab}	293^{ab}	82.1	247^{ab}	244^{ab}	180^{ab}	21.2	42.5	152
13: -NEA	1454^{a}	1179^{a}	76.7	46.0^{b}	1172^{a}	141^{a}	267^{a}	75.7	228^{a}	227^{a}	169^{a}	20.0	40.5	142
14: -½NEA	1542^{ab}	1257^{ab}	81.0	44.9^{ab}	1250^{ab}	147^{ab}	289^{ab}	79.0	244^{ab}	244^{ab}	179^{ab}	20.8	42.3	145
15: +½glu	1593^{b}	1308^{b}	80.7	44.2^{ab}	1301^{b}	152^{b}	309^{b}	83.5	257^{b}	247^{ab}	186^{b}	20.7	42.6	148
16: +glu	1566^{ab}	1296^{b}	79.5	37.1^{a}	1290^{b}	153^{b}	304^{b}	78.4	252^{b}	246^{ab}	185^{b}	22.3	43.8	138
17: +NEA	1576^{b}	1297^{b}	81.7	42.1^{ab}	1290^{b}	154^{b}	307^{b}	79.5	253^{b}	245^{ab}	185^{b}	20.0	43.3	142
F-prob.	0.018	0.004	0.630	0.047	0.003	0.009	0.002	0.532	0.002	0.012	0.033	0.609	0.161	0.568
sed	37.8	31.6	2.6	3.0	31.4	3.5	9.2	4.3	6.5	6.7	5.5	1.4	1.1	6.9

^{abc} Within a column, means with same letter are not significant different (t-test; $P<0.01$)

Legend of portions: Sla. = Slaughter weight, Oven = Oven ready weight, Org = Edible organs (liver, heart, gizzard), Fat pad = abdominal fat pad, Griller = Chilled carcass, Wings = Wings, Breast = Breast meat without skin, Skinfat = Skin plus skinfat, Carcass = remaining carcass after cutting other portions, Thighs = Thighs, Drums= Drums, Ne-sk = Neck skin, Neck = Neck, Tfat = Fat pad + Skinfat + Ne-sk

Relative to live weight prior to slaughter (table 4), only few differences in yield were caused by variation in NEAA content. Dressing efficiency (Oven ready weight as percentage of slaughter weight), griller percentage and percentage of remaining carcass were affected by variation in NEAA content. Dressing efficiency was lowest for the diet with 25 % reduced NEAA (13) and highest for the diet with 25 % extra Glu (16) relative to pattern of body accretion. The same observation was found for griller efficiency relative to live weight and for the remaining carcass. Although absolute weights of portions were affected by NEAA content, composition of the carcass was not affected by NEAA content.

Experiment 3: Effect of thr and NEAA content

In this experiment at first interaction between Thr and NEAA was tested. In case of a significant interaction the main effect of Thr or NEAA will only be discussed if this effect is independent of the interaction.

The absolute slaughter weights, as shown in table 5, revealed several significant (F-test; $P<0.005$) interactions between Thr and NEAA content (slaughter weight, oven-ready weight, griller, wings, breast meat and remaining carcass). No significant interactions but only main effects were found for abdominal fat pad, fat and skin, thighs, drums, neck and total fat. No statistical significant effects of either treatment were found for edible organs and neck skin. In all instances of interaction the effect of Thr level was lower or absent at low NEAA level and highest at high NEAA level. When interaction was not significant and a Thr effect was found, the low Thr level was different from the other two Thr levels. In case of a separate NEAA level effect it is either the high (abdominal fat pad, total fat) or the low NEAA level (thighs, drums) which differed from the other two. If present, the effect of both lowering Thr and NEAA levels on absolute weights was to decrease absolute weights.

When relative yields were considered (table 6), interaction was significant ($P<0.05$) for slaughter efficiency and griller efficiency. Separate effects of Thr level were found for dressing percentage, organs, breast meat, drums and skin of the neck. Separate effects of NEAA level were found for dressing percentage, abdominal fat pad and total fat.

Slaughter efficiency was only at low Thr level affected by NEAA level in the sense that high NEAA level resulted in a lower slaughter efficiency. At medium and high Thr level the effect of NEAA level was not significant. Or, alternatively, at low NEAA level lower Thr level resulted in higher slaughter efficiency whereas at

Table 4. Slaughter efficiencies experiment 2

Portion:	Sla.	Oven	Org	Fat pad	Griller	Wings	Breast	Skinfat	Carcas	Thighs	Drums	Ne-sk	Neck	Tfat
9: CC	85.4	84.7^{bc}	4.3	2.3	69.8^{b}	8.2	16.2	4.5	13.6^{ab}	13.6	10.0	1.1	2.3	8.0
10: -asp	85.2	84.8^{bc}	4.4	2.4	69.6^{ab}	8.3	16.2	4.4	13.5^{ab}	13.4	10.1	1.2	2.4	8.0
11: -ala	85.5	84.6^{bc}	4.4	2.4	69.8^{b}	8.2	16.0	4.4	13.6^{ab}	13.7	10.2	1.1	2.4	7.8
12: -glu	85.7	84.1^{ab}	4.5	2.7	69.5^{ab}	8.2	16.1	4.5	13.6^{ab}	13.4	9.9	1.2	2.4	8.4
13: -NEA	85.3	83.8^{a}	4.5	2.7	68.7^{a}	8.3	15.6	4.4	13.4^{a}	13.3	9.9	1.2	2.4	8.3
14: -½NEA	85.6	84.2^{ab}	4.5	2.5	69.5^{ab}	8.2	16.1	4.4	13.6^{ab}	13.6	9.9	1.1	2.3	8.0
15: +½glu	85.5	84.7^{bc}	4.3	2.4	69.9^{b}	8.2	16.5	4.5	13.8^{b}	13.2	10.0	1.1	2.3	8.0
16: +glu	85.3	85.3^{c}	4.3	2.0	70.3^{b}	8.3	16.5	4.3	13.8^{b}	13.4	10.1	1.2	2.4	7.5
17: +NEA	85.4	84.8^{bc}	4.4	2.3	69.9^{b}	8.3	16.6	4.3	13.7^{ab}	13.3	10.0	1.1	2.3	7.7
F-prob.	0.616	<.001	0.683	0.002	0.011	0.814	0.034	0.828	0.031	0.122	0.619	0.495	0.874	0.085
sed	0.275	0.307	0.136	0.149	0.352	0.110	0.293	0.182	0.123	0.154	0.163	0.075	0.062	0.281

^{abc} Within a column, means with same letter are not significant different (t-test; $P<0.01$)

Legend of portions: Sla. = Slaughter weight, Oven = Oven ready weight, Org = Edible organs (liver, heart, gizzard), Fat pad = abdominal fat pad, Griller = Chilled carcass, Wings = Wings, Breast = Breast meat without skin, Skinfat = Skin plus skinfat, Carcass = remaining carcass after cutting other portions, Thighs = Thighs, Drums = Drums, Ne-sk = Neck skin, Neck = Neck, Tfat = Fat pad + Skinfat + Ne-sk; all, except oven, relative to live weight; oven is relative to slaughter weight.

medium or high NEAA level Thr content did not affect slaughter efficiency. Griller percentage was at low NEAA level not affected by Thr content wheras at medium or high NEAA level low Thr level resulted in lower griller percentage.

Dressing percentage was significantly lower at low Thr level and was raised by higher NEAA level. Organs constituted a larger part of live weight in birds fed low Thr than in birds fed adequate or high Thr levels. Fat pad was higher when dietary NEAA level was lowered.

Discussion

As shown in the results section, dietary amino acid pattern affects slaughter yield, not only in an absolute way but also relative to live weight. This implies that distribution of tissue can be altered by dietary amino acid pattern. This change in tissue distribution could be due to priority given to development of functional organs or it could be caused by an inability to synthesize certain tissues or by changes in the non-functional storage of proteins. In general under condition of sub-optimal nutrient supply the organs are better developed than meat suggesting that organ development is given higher priority. Especially breast meat reacts favourably to an increased amino acid level in diets which suggests that breast meat is only developed to its potential after all other organs are already developed and there is still ample supply of amino acids. Since breast meat accounts for close to 50 % of edible protein yield Summers et al. (1988) suggested to use yield of edible breast protein as a measure of dietary protein utilisation. Holsheimer & Veerkamp (1991) similarly concluded that especially yield of breast meat reacted favourably to better amino acid (Lys) supply. In a succeeding paper Holsheimer & Ruesink (1993) observed no positive effect of a more than normal lysine supplementation in the second growth phase (15-49 d) on breast meat yield. It was suggested that dietary amino acid supplementation should be economically evaluated. The high yield of breast meat of birds fed diet 6 (1 + Met) suggests that extra methionine in the second part of the growth period also stimulates breast growth. Schutte & Pack (1993) concluded, based on the observation that a high Met level induced high yields of breast meat, that economic optimum for dietary Met level was higher if birds were sold for porcessing than when whole birds were sold.

It seems that slaughter efficiency is only slightly affected by dietary NEAA content. As shown in a previous publication (ten Doeschate, 1995a) absolute production is affected but highest nitrogen efficiency is attained with low NEAA

Table 5. Slaughter weights experiment 3

NEAA	thr	Sla.	Oven	Org	Fat pad	Griller	Wings	Breast	Skinfat	Carcas	Thighs	Drums	Ne-sk	Neck	Tfat
	L	1370^{ab}	1104^{ab}	76.2	41.8^{abc}	1098^{ab}	135^{ab}	249^{a}	70.1^{ab}	218^{ab}	216^{ab}	153^{a}	17.9	38.0^{a}	130^{ab}
Low	M	1454^{bcd}	1179^{bcd}	76.7	46.0^{c}	1172^{bcd}	141^{bc}	267^{ab}	75.7^{abc}	228^{abc}	227^{abcd}	169^{bc}	20.0	40.5^{ab}	142^{b}
	H	1468^{bcde}	1204^{cde}	76.8	40.2^{abc}	1197^{cde}	144^{bcd}	276^{bc}	75.1^{abc}	234^{bcd}	235^{bcde}	172^{bcd}	17.6	40.5^{ab}	133^{ab}
	L	1408^{abc}	1133^{abc}	78.7	43.4^{bc}	1127^{abc}	134^{ab}	251^{ab}	72.7^{ab}	224^{abc}	225^{abc}	158^{ab}	20.8	37.9^{a}	137^{ab}
Med.	M	1605^{f}	1318^{f}	80.6	44.1^{bc}	1312^{f}	155^{d}	304^{d}	85.3^{c}	256^{e}	256^{e}	188^{e}	20.1	43.1^{b}	150^{b}
	H	1523^{cdef}	1251^{def}	79.1	41.7^{abc}	1244^{def}	149^{cd}	295^{cd}	77.0^{bc}	240^{cde}	239^{cde}	179^{cde}	18.9	42.4^{b}	138^{b}
	L	1330^{a}	1077^{a}	76.7	33.8^{a}	1071^{a}	129^{a}	241^{a}	64.7^{a}	212^{a}	212^{a}	150^{a}	18.7	36.8^{a}	116^{a}
High	M	1566^{def}	1296^{ef}	79.5	37.1^{ab}	1290^{ef}	153^{cd}	304^{d}	78.4^{bc}	252de	246^{cde}	185de	22.3	43.8^{b}	138^{ab}
	H	1574^{ef}	1296^{ef}	82.7	37.3^{ab}	1289^{ef}	154^{d}	305^{d}	81.9^{bc}	252de	247de	182^{cde}	20.0	43.8^{b}	139^{b}
F-prob NEAA		0.009	0.003	0.199	<.001	0.003	0.043	<.001	0.160	0.003	0.011	0.025	0.088	0.084	0.048
sed		25.5	20.8	1.9	1.8	20.7	2.5	5.3	2.5	4.0	4.6	3.7	0.9	0.8	4.2
F-prob thr		<.001	<.001	0.450	0.218	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.062	<.001	0.002
sed		25.5	20.8	1.9	1.8	20.7	2.5	5.3	2.5	4.0	4.6	3.7	0.9	0.8	4.2
F-prob		0.047	0.026	0.692	0.571	0.025	0.027	0.008	0.121	0.011	0.068	0.246	0.318	0.177	0.215
sed		44.2	36.1	3.2	3.0	35.9	4.3	9.3	4.3	7.0	8.0	6.4	1.5	1.44	7.2

^{abc} Within a column, means with same letter are not significant different (t-test; $P<0.01$)

Legend of portions: Sla. = Slaughter weight, Oven = Oven ready weight, Org = Edible organs (liver, heart, gizzard), Fat pad = abdominal fat pad, Griller = Chilled carcass, Wings = Wings, Breast = Breast meat without skin, Skinfat = Skin plus skinfat, Carcass = remaining carcass after cutting other portions, Thighs = Thighs, Drums = Drums, Ne-sk = Neck skin, Neck = Neck, Tfat = Fat pad + Skinfat + Ne-sk

levels. Although total production is decreased with decreased dietary NEAA relative slaughter yields are not affected. This implies that relative proportions of muscle, bones and organs are not affected. No effects on absolute weights of abdominal fat pad or total skin plus fat were found. However, relative to body weight high NEAA diets decreased abdominal fat pad. This is in accordance with data of Moran et al. (1992) and Leclerq et al. (1994). An explanation for this can be found in the fact that relative to growth, in low NEAA diets dietary intake was higher and thus also relative to growth energy intake was higher. This extra energy was then deposited as fat.

The interactions between dietary NEAA level and Thr content show that the negative effect of low Thr content is lower in low NEAA diets than in high NEAA diets. Especially for griller as percentage of live weight it is clear that at low NEAA level there is no negative effect of low Thr level whereas at higher NEAA levels low Thr level results in a decrease in griller yield. This observation is in accordance with the hypothesis forwarded in the introduction that high dietary NEAA level results in extra utilization of Thr for the conversion to glycine in order to allow uric acid formation and thus in a relative shortage of threonine. The observation that absolute organ weights were not affected by either Thr or NEAA level suggests that these organs (heart, gizzard and liver) have a priority in growth.

Conclusions

Based on the present paper three main conclusions can be drawn:

1) Variation in the dietary essential amino acid pattern, within one of three proposed amino acid patterns, has only minor influence on slaughter efficiencies.
2) Variation in dietary NEAA level has no effect on relative contribution of the separate body parts to the whole body.
3) There is a distinct interaction between dietary Thr and NEAA level on body constituents which indicates that dietary Thr requirements are higher in case of a high NEAA diet.

Table 6. Slaughter efficiencies experiment 3

NEAA	thr	Sla.	Oven	Org	Fat pad	Griller	Wings	Breast	Skinfat	Carcass	Thighs	Drums	Ne-sk	Neck	Tfat
	L	86.3^{b}	83.3^{a}	4.8^{bc}	2.6^{b}	69.2^{ab}	8.5	15.7^{abcd}	4.4	13.8	13.6	9.6^{a}	1.1^{abc}	2.4	8.1
Low	M	85.3^{a}	83.8^{a}	4.5^{abc}	2.7^{b}	68.7^{a}	8.3	15.6^{abc}	4.4	13.4	13.3	9.9^{ab}	1.2^{abc}	2.4	8.3
	H	85.3^{a}	84.6^{bc}	4.5^{abc}	2.3^{ab}	69.5^{abc}	8.4	16.1^{abcd}	4.4	13.6	13.6	10.0^{ab}	1.0^{a}	2.4	7.7
	L	85.7^{ab}	83.2^{a}	4.8^{bc}	2.6^{b}	68.5^{a}	8.2	15.2^{a}	4.4	13.6	13.7	9.6^{a}	1.3^{c}	2.3	8.3
Med.	M	85.4^{a}	84.7^{bc}	4.3^{a}	2.3^{ab}	69.8^{bc}	8.2	16.2^{bcd}	4.5	13.6	13.6	10.0^{ab}	1.1^{ab}	2.3	8.0
	H	85.5^{ab}	84.7^{bc}	4.5^{ab}	2.4^{ab}	69.8^{bc}	8.4	16.5^{cd}	4.3	13.5	13.4	10.0^{ab}	1.1^{ab}	2.4	7.7
	L	85.0^{a}	83.9^{ab}	4.9^{c}	2.1^{a}	68.5^{a}	8.3	15.4^{ab}	4.1	13.6	13.5	9.6^{a}	1.2^{bc}	2.4	7.4
High	M	85.3^{a}	85.3^{c}	4.3^{a}	2.0^{a}	70.3^{c}	8.3	16.5^{cd}	4.3	13.8	13.4	10.1^{b}	1.2^{bc}	2.4	7.5
	H	85.3^{a}	84.9^{c}	4.5^{ab}	2.0^{a}	69.9^{bc}	8.3	16.6^{d}	4.4	13.6	13.4	9.9^{ab}	1.1^{ab}	2.4	7.5
F-prob NEAA		0.045	0.002	0.676	<.001	0.075	0.205	0.165	0.328	0.437	0.400	0.957	0.220	0.493	0.004
sed		0.161	0.211	0.09	0.097	0.185	0.07	0.188	0.109	0.084	0.092	0.112	0.041	0.045	0.180
F-prob thr		0.147	<.001	<.001	0.069	<.001	0.457	<.001	0.495	0.630	0.276	0.003	0.006	0.758	0.264
sed		0.161	0.211	0.09	0.097	0.185	0.07	0.188	0.109	0.084	0.092	0.112	0.041	0.045	0.180
F-prob NE*thr		0.006	0.195	0.784	0.429	<.001	0.405	0.064	0.566	0.123	0.272	0.847	0.146	0.776	0.467
sed		0.280	0.366	0.155	0.169	0.321	0.122	0.326	0.189	0.146	0.159	0.194	0.071	0.078	0.312

[abc] Within a column, means with same letter are not significant different (t-test; $P<0.01$)

Legend of portions: Sla. = Slaughter weight, Oven = Oven ready weight, Org = Edible organs (liver, heart, gizzard), Fat pad = abdominal fat pad, Griller = Chilled carcass, Wings = Wings, Breast = Breast meat without skin, Skinfat = Skin plus skinfat, Carcass = remaining carcass after cutting other portions, Thighs = Thighs, Drums = Drums, Ne-sk = Neck skin, Neck = Neck, Tfat = Fat pad + Skinfat + Ne-sk; all, except oven, relative to live weight; oven is relative to slaughter weight.

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GENERAL DISCUSSION

Introduction

In this thesis the development of a physiological feeding strategy for protein in broilers has been described. Several aspects have been discussed. In this final chapter the main points of the previous chapters will be discussed in a more integrated approach. Moreover, some of the subjects will be further elucidated. Based on the conclusions of the study the practical implications of the physiological feeding strategy will be discussed. Subsequently I will discuss the possible environmental consequences of aiming for high N-efficiency of broiler production in the Netherlands. This subject is not strictly part of the physiological feeding strategy but I feel it important to discuss this aspect because it places this study in it's social context. Finally, suggestions for further research will be given.

Integration of previous chapters

The concept of a physiological feeding strategy was that adjustment of dietary composition to physiological demands would result in the highest efficiency of amino acid deposition. As discussed in the introduction, efficiency is no feature of protein deposition but it is the arithmetical result of different metabolic processes. Within the physiological feeding strategy it was intended to let the diet supply the amino acids necessary for the required processes but to prevent excess amino acid supply in order to diminish amino acid catabolism. For this strategy it is necessary to know the required amino acid pattern in the diet at different stages of growth and the availability of amino acids from the diet. As discussed in the introduction, requirements can be determined in classic requirement studies or by a factorial

approach. For broilers the factorial approach is based on the amino acids required for nett protein deposition combined with amino acids required for non-productive purposes. In this factorial approach the amino acid pattern of body accretion was used as basis for requirements. It was to be expected that the required amino acid pattern would differ for chicks of different ages since relative growth differs with time between different body tissues. For this reason the first experiment was aimed to define the changes in the amino acid pattern of body accretion during the development from day-old chick to heavy broilers at slaughter age (7 wks). It was shown that some changes in whole body amino acid pattern are statistically significant but still relatively small (Table 3, chapter 1). Furthermore, the whole body amino acid pattern of chicks of different genotypes as well as female and male chicks was determined. Birds of different genotypes and sexes may have a different body composition due to differences in sizes of organs and muscles. Differences between genotypes or between sexes in amino acid pattern were statistically significant but also small (Table 4 and 5, chapter 1). When the amino acid pattern of body accretion is taken as basis for diet composition this would imply that, at least based on this study, birds of different genotype, sex and age would require a different dietary amino acid composition. The changes with age were more pronounced than the differences between sexes or genotypes. The female amino acid pattern contained in general relatively more non-essential and less essential amino acids. Within the essential amino acids the pattern was similar for both sexes. Because of these observations it was concluded that it would be possible to base the dietary amino acid composition on the amino acid pattern of body accretion of male chicks. It was expected that the resulting dietary imbalance between essential and non-essential amino acids in female chicks would not result in a significant decrease in performance. In chapter 5 and again in chapters 6 and 7 it was shown that the amino acid pattern of body accretion could be used as a basis for diet composition. There were no significant differences in N-efficiency between diets based on the amino acid pattern of body accretion and diets based on the required amino acid pattern according to Baker & Han (1993). In chapter 5 it was shown that also birds of the FC genotype (birds selected for favourable feed conversion ratio) reacted similar to a change in diet composition as did Com birds (a commercial broiler hybrid). No indication was found that the dietary amino acid pattern for female birds or birds of the FC genotype should differ from that defined for male Com birds. This result implies either that differences in amino acid pattern of body accretion between genotypes were so small that the adjustments of dietary levels were not necessary or that performance was limited by the same limiting amino acid in both cases. The latter could be the case but it is rather unlikely that

in all cases a similar restriction in performance would occur, even if a single amino acid would be limiting in the diet. Moreover, the differences in amino acid pattern required for body accretion in the different kinds of birds should result in different limiting amino acids when fed an identical amino acid pattern.

In all genotypes it was found that the lowest protein/energy ratio (P/E-ratio) resulted in the highest N-efficiency. However, growth was decreased. There was no interaction between P/E ratio and supplementation with a mixture of Met, Arg, Glu and Gly. This implies that at none of the three levels of P/E ratio there was a limitation due to low levels of either Met or Arg or non-essential amino nitrogen. The increase of N-efficiency with lower P/E ratio indicates that the lowest P/E ratio used in this experiment gave a sufficient protein supply or in other words: at the higher P/E levels the supply of extra protein relative to the energy supply resulted in a limited utilisation of amino acids for protein synthesis. The amount of diet eaten is, in birds, supposed to be related to the energy input. It is important to realize that maximal nitrogen efficiency can only be achieved when energy supply relative to amino acid intake is sufficient. A possible drawback of a generous energy supply relative to protein intake is an increase of fat content in the birds. Concerning P/E ratio the observations in chapter 5 (Tables 3 and 7) lead to the conclusion that the lowest P/E ratio used in the experiment results in the highest N-efficiency. However, with respect to other performance parameters the middle P/E ratio might be preferred.

The similar reaction of different genotypes to amino acid supplementation (chapter 5) implies that the differences in body amino acid pattern had no effect on the required amino acid composition. This can be explained when the differences are examined more thoroughly. Differences in whole body amino acid pattern were mostly found in non-essential or conditional essential amino acids. The essential amino acids were present in almost equal quantities in all birds. Therefore, the pattern of essential amino acids is similar for all birds. When equal metabolic utilisation of amino acids is assumed, this implies that the amino acid pattern of male commercial type broiler chicks can be used as basis for the dietary amino acid pattern for all types of broilers. Only in case of differences in metabolic amino acid utilisation of the essential amino acids, i.e. differences in non-productive requirements between genotypes and sexes different dietary amino acid patterns should be used for different genotypes and sexes.

In chapter 6 and 7 it is shown that a further increase of N-efficiency could be attained by a reduction of the non-essential amino acid content of the diet. This approach was based on the hypothesis that the N from degraded amino acids is

transformed to non-essential amino acids prior to uric acid synthesis. If these non-essential amino acids (Glu + Ala + Asp, NEAA) could be utilized for nett protein synthesis instead of uric acid synthesis this would result in an increase in N-efficiency. This concept, as tested in chapter 6 and 7, proved to be valid. However, the increase in nitrogen efficiency with low dietary NEAA content was accompanied by a decrease in growth rate and an increase in feed conversion ratio. It can be concluded that the hypothesis that lowering dietary NEAA level might increase N-efficiency is correct but probably not without restrictions. The metabolic limits to this approach have to be determined in more detail. For practical application also an economic analysis has to be made.
The highest possible nitrogen efficiency theoretically will be reached if non-productive utilisation of amino acids is kept as low as possible. It has been shown in chapter 3 and 4 that oxidation can be affected by a variety of circumstances. Perturbation of the metabolic utilisation of amino acids will lead to extra oxidation and thus extra uric acid excretion. For this reason birds should be in a steady growing state with frequent food intake. Thus, supply of nutrients should be directed to enable birds to express their potential for most efficient protein deposition. This is just what a physiological feeding strategy should be based upon.

Use of the amino acid pattern of body accretion for diet formulation

In the first chapter the development of the amino acid pattern in the body with increasing age of the broiler and thus in body accretion is described. Conclusions drawn in this chapter were that amino acid composition of the whole body is fairly constant. Nevertheless separate phases for amino acid pattern of body accretion could be distinguished. In chapter 6 and 7 the amino acid pattern of body accretion was used to compose an experimental diet. This diet was compared to diets composed either according to requirements given by Baker & Han (1993) or to resemble a practical diet. Additionally some diets with variation in dietary composition were used in order to test whether single differences in amino acid pattern were the cause of possible differences in performance on the diets. The diet based on the amino acid pattern of body accretion gave approximately the same results as the diet based on Baker & Han (1993). The practical diet resulted in slightly better growth and feed conversion ratio but nitrogen efficiency was lower. From the results of the slaughter experiment (chapter 7) similar conclusions can be drawn. Production of edible meat per unit of nitrogen intake was highest for both the Baker diet and the diet based on the amino acid pattern of body accretion. In

these experiments it was also shown that the high histidine level found in body accretion could be lowered down to the level according to the Baker pattern without negative effects on any performance parameter. In general it can be concluded that the amino acid pattern of body accretion can be used for diet formulation. Adjustments for non-productive requirements do not appear to be very necessary. This can be explained by the high growth rate of broilers which results in a relative large contribution of the amino acids required for body accretion on total requirements. As shown in chapter 6, seventy percent of available nitrogen is deposited in the body which substantiates the claim that required amino acid pattern is mainly based on the amino acid pattern of accretion. Moreover, the 30 % of amino acids not deposited is available to cover non-productive requirements. The choice to express the dietary supply of amino acids on an apparent ileal digestible basis helps also to ensure that requirements are mainly determined by requirements for body accretion. The reason for this is that a part of non-productive requirements, the loss due to digestive processes, are already accounted for in the digestibility of the diet. This will be discussed a bit further in a following paragraph. We conclude that the amino acid pattern of body accretion is a good basis for diet formulation.

Relevance of feeding in phases

Phase-feeding has been seen as a way to adjust the diet composition during a given period as good as possible to the requirements of the animals. It is, however, questionable whether phase-feeding is the optimal answer to changes in required diet composition as birds age. Firstly, changes in requirement are not likely to occur suddenly during development of a bird but are more likely to be the result of gradually changing processes. Furthermore, the time span of development may differ between birds which implies that, given an abrupt change in requirements would exist, not all birds would need this change at the same moment. Because of these two statements one would prefer a gradual change in diet composition instead of an abrupt one. On some farms nowadays a so-called multi-phase feeding programme is used. In this kind of program on the farm a mixture is made of two basic diets. The ratio is gradually changed each day. This can be seen as a gradual change of diet composition opposed to a sudden change. In research this is hard to use since a daily change of ration is hardly feasible in small scale units such as a pen with 10 to 15 birds. However, the multi-phase system might offer

advantages since the change in diet can be made more gradually with more birds being fed approximately adequately.

It was shown in chapter 1 that the whole body amino acid pattern changed gradually but that the changes were relatively minor. Thus, changes in composition of body accretion do not dictate distinct feeding phases. However, the observed changes in the pattern were statistically significant which justifies some attention to differences in dietary amino acid pattern in separate growth phases. In practical broiler feeding a feeding system with two or three consecutive diets is generally accepted. Until now, this is mainly used to adjust dietary P/E ratio and energy level to the birds' requirements. The first diet, also known as the starter diet, is characterised by a high protein/energy ratio, contains not to much fat and is highly digestible. The second or grower diet has a lower protein/energy ratio, has a higher energy level and may contain more fat and more difficult to digest ingredients. The last or finisher diet is rather similar to the grower diet although it may have a lower protein/energy ratio and a higher energy level but the main difference with the grower diet is that it does not contain feed additives. Variation in amino acid pattern between the phases is rather limited. This is not only due to lack of knowledge concerning requirements but also to the practice that in diet formulation only the contents of the first limiting amino acids (Met + Cys, Lys, Thr and Trp) are taken into concern. Methionine relative to lysine is set at a higher level in grower than in starter diets but for the other amino acids no changes are made. So, in practice, feeding different diets in different phases is mainly used to control P/E ratio and energy level but not to adjust dietary amino acid pattern to changes in amino acid requirements with age. The amino acids, other than the limiting ones mentioned before, are supposed to be available in excess when a 'normal' diet with a sufficient high protein level is used. A practical conclusion is: It is suggested to adjust the dietary amino acid pattern according to the changes in the amino acid pattern of body accretion as observed in the phases used in practice (two or three phases). For a multiphase system it would be ideal to have the amino acid pattern of the diet to change gradually according to the changes found in body accretion.

How important is digestibility of foodstuffs for a physiological feeding strategy ?

Digestibility is important since the physiological feeding strategy is focused on the metabolic utilisation of amino acids available from the diet. In classic digestibility studies, digestibility is measured at the droppings level. This might cause errors in

the estimated availability due to changes in amino acid composition of the intestinal contents between the place whereafter absorption of amino acids is no longer possible and the sampling of the droppings. For this reason digestibility should be measured at the point where absorption of amino acids stops which, according to Webb (1990), in chicks is in the terminal ileum. For this reason an ileal sampling technique was used. Since it is known that digestibility differs between adult and young birds this technique should be performed in normal growing, young birds. This implies that the use of anatomically altered birds (e.g. colostomized) or birds fitted with an ileal canula is not advised. Based on the research of van der Klis et al. (1992) a slaughter method was used of which the results are presented in chapter 2.

We would like to know the nett absorption of amino acids from the diet. In this way, the loss of amino acids with digestive enzymes and wear of the intestinal lining (mucosa) is taken as a part of digestibility and not as a non-productive requirement. This makes non-productive requirements less important for the total required amino acid pattern since Fuller (1991) stated that the largest part of maintenance requirements (= part of non-productive requirements) was the amino acids lost in the digestive process. When an apparent digestibility is taken as a measure of amino acid availability this loss is already accounted for. So, in our opinion an apparent digestibility would be better than a 'true' digestibility.

Digestibility studies are so basal for the calculation of the available amino acids that we thought it appropriate to include a study on digestibility in broiler chicks in the present thesis. The results of Scheele et al. (1992) who performed digestibility studies in a way similar to that described in chapter 2, were used to calculate ileal digestible amino acid contents for the diets used in the experiments described in chapter 3 till 8.

What is the significance of metabolic amino acid utilisation studies in broilers ?

The results of the metabolic studies, as described in chapter 3 and 4, indicated that the oxidation of amino acids is affected by several physiological conditions. The conditions investigated were feeding status, age, diet fed and length of the period of conditioning to the diet. However, besides the investigated influences there are several extra influences since, although conditions were kept as constant as possible, there was a large variation in the obtained results. This variation indicates that metabolic amino acid utilisation in broilers is also affected by conditions other than the ones investigated. Possible reasons for this extra variation are: genetic differences between chicks, stress effects, variation in oxidation over the day,

variation due to measuring technique etc. The sensitivity of oxidation for various physiological factors can be seen as a drawback in that it makes metabolic amino acid utilisation studies less useful for measurement of small dietary effects. However, it can also be seen as an opportunity to learn more about the way amino acid metabolism works. It may be clear that the high variation in measured oxidation of amino acids is a sign that such short term variation is inherent to the chick. For a variety of metabolic reasons, oxidation will always take away a certain part of the free amino acids from protein synthesis. In future research it could be investigated to which extent physiological conditions, other than diet, feeding regime and conditioning, affect metabolic amino acid utilisation. Research in rats (Schreurs & Koopmanschap, 1995) has shown that so-called metabolic perturbations cause increased oxidation. Another interesting question is the question whether all amino acids are equally sensitive to metabolic disturbances. Schreurs & Koopmanschap (1995) indicated that at least in the rat lysine would be less sensitive than leucine.

The sensitivity for metabolic disturbances might explain some observations concerning time-lag effects when feeding free amino acids combined with intact proteins. For supplements of lysine and methionine time-lag effects are not considered a problem but when more and other amino acids are fed as free amino acids time-lag effects seem to occur (Bach Knudsen & Jørgensen, 1986). This might be caused by differences in sensitivity to metabolic disturbance. However, time-lag effects can only occur when food intake is not evenly spread over the day or, more precise, when feed intake is relative to metabolic utilisation discontinuous. In broiler chicks feed intake is mostly well spread over the day. Therefore we do not expect serious problems with time-lag in broilers, unless feed intake is not evenly spread over the day. Problems might however occur in case of restricted feeding, alternative lighting schedules (such as 16L:8D) or other management conditions resulting in discontinuous feed intake. The sensitivity of amino acid metabolism to metabolic perturbation indicates whether a time-lag results in an increase in amino acid oxidation. In conclusion, the variation in $^{14}CO_2$ recovery from injected [^{14}C]amino acids mainly leads to extra insight in consequences of short term changes in metabolism of amino acids. This may contribute to a better understanding of differences in consequences of time-lag effects between amino acids.

The results described in chapter 4 when diets differing in Met content were fed were interpreted as an example that the use of indicator amino acids to determine

amino acid requirements can be especially useful for requirements which vary on a relative short term basis (± a week). It was shown that in the fourth week of age oxidation of an indicator amino acid (Leu) reacted to dietary methionine level. In birds fed the low Met diet $^{14}CO_2$ recovery from [^{14}C]Leu was higher than in birds fed high the Met diet. Thus, in birds fed the high Met diet a larger part of Leu flux was deposited than when a low Met diet was fed. This can be interpreted as that Met requirement was not met at the low Met level. In other periods there were no differences between any dietary Met level found which implies that in other periods the low Met level was sufficient whereas in the fourth week of age Met requirement was increased. This might be caused by the relative high requirement for sulphur containing amino acids for feather formation in this period. This kind of variation in requirements during development of broilers is ideal to be studied with the $^{14}CO_2$ breath test technique. Buten (1989), who studied protein metabolism of broiler chicks after feeding different methionine supplements, concluded that tracer studies were complicated and as expensive and time-demanding as simple growth assays but that only knowledge of differences on a molecular basis would lead to reliable valuation of methionine supplements. Based on the present data this conclusion can be supported. Metabolic utilisation studies are complicated but they do provide insight to what is really happening in amino acid intermediate metabolism. The value of metabolic amino acid utilisation studies lies thus in the better understanding of amino acid metabolism. Especially the short term relation between availability of amino acids and capacity to utilize these for protein synthesis may provide opportunities for improvement of amino acid utilisation.

Possibility to increase N-efficiency by a decrease in dietary NEAA content.

In the introductory chapter it was hypothesized that a decrease of dietary NEAA content could possibly result in a higher nitrogen efficiency. The basis for this hypothesis was that metabolic degradation of amino acids results in transfer of nitrogen from degraded amino acids to Glu, Asp and Ala in order to transport the amino acid N from the site of de-amination to the site of uric acid synthesis. In the experiment described in chapter 6 and 7 this hypothesis was tested. It was shown that nitrogen efficiency could be increased by decreasing dietary NEAA content but only with a simultaneous negative effect on growth and feed conversion ratio. Apparently these NEAA formed in the metabolic degradation of other amino acids are not as readily available for protein synthesis as expected based on the constituents of uric acid (Figure 1). The excretion of uric acid proceeded, indicating

that uric acid synthesis was not impaired while nett protein synthesis was lower than could be attained on a diet with a normal NEAA content. This problem is probably due to NEAA deficiency at the protein synthesis sites. This points to a problem in the allocation of the NEAA within the organism. A reason for this could be that a large part of amino acid degradation is located in the liver while also uric acid synthesis is located in the liver. Thus, when low NEAA diets are fed circulating levels of NEAA levels might be too low for maximum protein synthesis. Unfortunately, no measurements of circulating amino acid levels were made in the present experiments. However, in a previous experiment, where two diets differing in Lys content were compared, free amino acid levels in bloodplasma were measured (ten Doeschate et al., 1993). The levels, relative to the total amount of free amino acids, of Asx (Asp + Asn), Ser, Gly, Ala, Cys, Met, Leu, Tyr, Phe, His and Pro were not significantly affected by diet. Thr, Val, Ile and Arg were higher

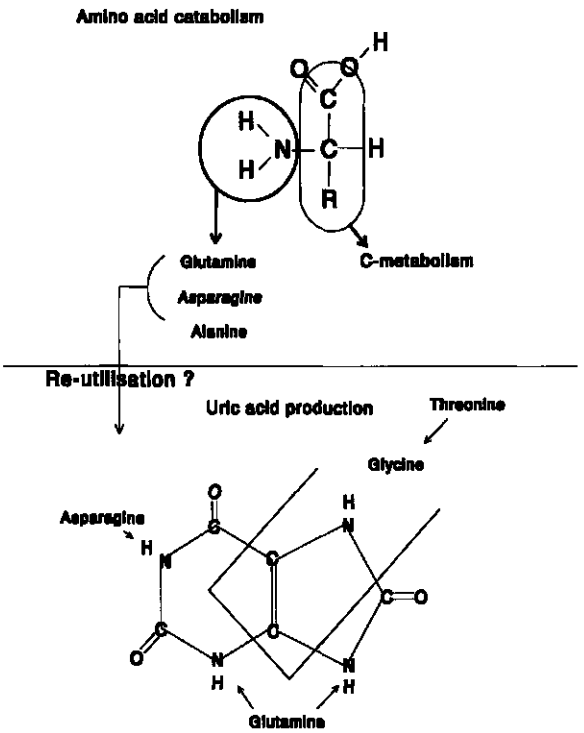

Figure 1. Contribution of N from non-essential amino acids, synthesized as a result of degradation of amino acids, to uric acid synthesis.

on the low-Lys diet whereas Glx (Glu + Gln) and Lys were lower on the low-Lys diet. On the low-Lys diet protein synthesis is limited by the low availability of lysine. This would result in extra degradation of other amino acids due to a relative excess. This, however is not reflected in a higher level of the products of amino acid degradation such as Glx and Asx. In fact, the level of Glx is even lower when the low-Lys diet is being fed. This suggests that excess amino acid degradation is mainly located in the liver and uric acid is synthesized immediately. Thus plasma Glx level is not increased.

There is still a severe lack of knowledge concerning this part of nitrogen metabolism in poultry. For instance it is not fully known what triggers the synthesis of uric acid. Is this process stimulated by the Glu level, Asp level, Gly level or some other metabolic factor. This would be of interest because apparently even in our experiment where Asp + Glu + Ala were decreased still uric acid was synthesized in a too large quantity because protein synthesis was impaired by low NEAA supply. On a whole body basis there is enough Glu + Ala + Asp but apparently at protein synthesis sites the level of these amino acids is lower than necessary for maximal protein synthesis. The problem thus is not a problem of an absolute shortage but merely a distribution problem. So, within the animal the problem is similar as on a global scale: There is not an absolute lack or excess of nitrogenous compounds but due to distribution there are local excesses and shortages. This implies that the one-pool model of amino acid as described in the introduction is clearly a simplification. There are sub-compartments which results in local deficiencies and excesses. Although in the present experiments the lower dietary NEAA level resulted in higher N-efficiency the decrease in growth rate and other performance parameters showed that further research on this subject is necessary. If the problem within the animal can be solved also a part of the global problem can be solved.

Practical implications of the present study

It is shown that adjustment of dietary composition results in an increase in N-efficiency. Firstly, use of the amino acid pattern of body accretion as basis for diet formulation results in a high N-efficiency. Secondly, low or medium P/E ratio increases N-efficiency compared to high P/E ratio. Thirdly, decrease of dietary NEAA level increases N-efficiency. However, this increase is often accompanied by adverse effects on growth rate and/or feed conversion ratio. As long as a higher nitrogen excretion has no immediate relation to cost, there is no stimulation to

formulate practical diets to increase nitrogen efficiency. As shown in chapter 6 and 7 the need to excrete nitrogen might result in an increased demand for Thr at a constant Gly level. This might stimulate the industry to be aware of the nitrogen excess in the diet to be excreted. The problem of interaction between dietary N-excess and Thr requirement can be tackled in three ways. The first and environmentally best solution would be to minimize dietary nitrogen excess in diet formulation. The second way, which leads to extra N-excretion, is to increase dietary Gly level in order to meet the increased Gly demand. As a third solution, which also increases N excretion but is more expensive than Gly supplementation, the Thr level can be increased. The first way might be most cost efficient and certainly reduces N excretion. In our opinion the first option would be the one to pursue as long as this can be attained within choice of normal foodstuffs.
The measured improvement in N-efficiency, in chapter 5 relative to a control diet and in chapter 6 relative to a 'practical' diet was between 4 and 8 %. In chapter 5 it was shown that, combined with an improved diet, birds selected for a low feed conversion ratio reached an efficiency of 124 % relative to normal broilers fed the control diet. In chapter 5 a calculation was made of the N-excretion relative to the control diet. It was shown that improvement of diet composition resulted in a relative nitrogen excretion of 89 % of control whereas the same diet fed to FC chicks resulted in a relative excretion of only 67 % of the excretion of Com birds when fed the control diet. From these results it may be concluded that improvement of nutrition as well as breeding for efficiency improves nitrogen efficiency. The practical implication of this observation is that breeding for N-efficiency is also worthwhile and that the effects of breeding and improvement of nutrition are additional.

An important practical implication of this study will be the answer to the question whether it is possible to reduce nitrogen excretion sufficient to meet the political limit of 50 % reduction. Firstly this depends on the starting-point of the calculations. It may be that the present practical diet has already been improved relative to the diets used in practice at the starting point of the project so compared to the reference point N-excretion might already be lowered. This means that the present improvement in N-efficiency is extra over the improvement attained by the feed industry. Secondly, improvement in nutrition is not the sole solution to the problem of excess nitrogen excretion. In the long run the aim is to have a steady state for the minerals in the agricultural areas. This means that enough but not too much minerals should be applied to the fields in order to compensate for inevitable losses and utilisation by the plants. One of the means to reach this aim is an

improved nutrition. Some other means are genetic improvements, better handling of manure, export of minerals and if all other means fail, reduction of animal production. However, as long as artificial nitrogen fertilizer is used in plant production, animal production can not be held solely responsible for a nitrogen excess.

Environmental consequences of aiming for high N-efficiency in the Netherlands

It should be realised that a change in diet composition in order to improve nitrogen efficiency of animal production does not necessarily result in a lower environmental burden. The explanation for this is that the aim of improving nitrogen efficiency of animal production might lead to a choice of better digestible ingredients . Eventually this would result in a lower utilisation of so-called by-products, debris of human food production. The utilisation of these by-products for animal nutrition can be seen as a delay in disposal. Instead of having to dispose of the debris it is used as foodstuff and only the manure of the animals is left as debris. When foodstuffs of higher quality are used in order to increase efficiency then animal production will compete more with human nutrition. So the idea to stimulate farmers for efficient use of nitrogen in the diet for animal production might work contra-productive on a global scale. Still, in the Netherlands the effect might be favourable since most animal foodstuffs are imported. If by-products are no longer imported then obviously the environmental burden will be less. However, on a global scale use of the available by-products for feeding the animals which can utilise them most efficiently will reduce environmental burden. The major advantage of Dutch intensive animal production has always been that, due to the proximity of Rotterdam as a main port, foodstuffs from all over the world can be combined to constitute the most economic complete diet. The best solution to the present problems in the Netherlands would be, besides measures in nutrition, breeding and management of manure, to export the excess minerals as fertiliser.

An additional observation from the experiments described in this thesis was that formulation of a diet to contain just the right amount of amino acids, for instance according to the pattern of body accretion, was impossible without ample use of free (synthetic) amino acids. The negative environmental impact of production of free amino acids might be as high as the reduction in pollution due to better efficiency of N-transfer from diet to animal. Data concerning the pollution due to and energy cost of amino acid production are however not available. This aspect may be important but as long as free amino acids can cost-effectively be used to

increase N-efficiency they will be used. In future the environmental pollution due to amino acid production will in some way be reflected in the price of amino acids. As long as the addition of free amino acids to a diet results in an increase in efficiency larger than the pollution caused by production of the amino acid the addition is from an environmental point of view justified.

Suggestions for further research

The chapter about body composition showed changes in composition with age, genotype and sex but did not show why these changes occurred. This could be further investigated by analysis of growth and composition of organs and separate parts of chicks. This subject will be looked into in the near future which might provide opportunities to stimulate growth of specific organs or body components.

The digestibility studies described in chapter 2 have lead to the development of a table of ileal digestible amino acids for broilers (Scheele et al., 1992). Subsequently Dijkslag et al. (1995) performed some experiments concerning the sensitivity of the assay to changes in experimental setting. It was shown that apparent ileal N-digestibility was not affected by dietary cellulose level (up to 15 %) nor by time between removal of the ileum and sampling of chyme (1 to 10 min). A difference was found between birds anesthesized or killed in that anesthesized birds had a higher apparent N digestibility. For practical application of apparent ileal digestibility as parameter to evaluate foodstuffs it would be necessary to perform experiments to compare diets formulated based on different assessment systems. As long as such experiments are not performed the choice for a method should be based on scientific arguments. As argued before, the ileal digestibility method as described in chapter 2 is favoured above other methods since amino acid absorption is supposed to stop at the end of the ileum and it is possible to measure ileal digestibility in normal, fast growing birds. The method of faecal digestibility only holds if it would give the same ranking and relative differences between foodstuffs as the ileal method.

In chapter 3 and 4 the use of $^{14}CO_2$ breath test measurements to study metabolic amino acid utilisation in broilers was described. It is shown that metabolic amino acid utilisation varies with several known and unknown physiological conditions. For further research I would suggest to try to find out how and why metabolic amino acid utilisation is influenced by several physiological factors. One way to get

a grip on this subject might be to study parts of the body instead of whole body studies. I would suggest to perform studies on isolated organs or tissue's in which substrate availability can be varied as intended and conditions can be kept more constant. This kind of studies might also provide more insight into the regulation of metabolic utilisation at organ or tissue level. The $^{14}CO_2$ breath test as such can be used to study amino acid metabolism in good defined birds to study whole body utilisation. Use of different labels simultaneously (^{13}C, ^{15}N) might give additional insight in utilisation of specific parts of the amino acids. However the use of stable isotopes brings about other problems due to larger doses required to attain minimum detection levels (Schreurs et al., 1991). Research in this field is very interesting for the understanding of amino acid metabolism. I think the only way to achieve progress in nutrition is by understanding the mechanism by which nutrients are utilised in the metabolism and why in metabolism certain choices are made.

Proceeding on this line I think that the classical approach of determining amino acid requirements will not provide us with major improvements in nutrition. Only deeper metabolic insight might improve the knowledge about proper nutrition. This, however, might not necessarily affect practical nutrition as it is nowadays. It might be that broiler feeding is already so sophisticated that major improvements are just not possible. The problem with present available knowledge is that limited information is available on the reaction to over- or undersupply and on interactions between nutrients. For this reason a more mechanistic model of broiler feeding is required. Attempts for modelling of broiler production have been made (e.g. Fisher, 1993) but usually the resulting models are not sufficient to predict performance when given dietary composition, capacity of the birds and several management conditions. During the development of such a model the points where knowledge is lacking are easily identified and thus research efforts can be steered in the right direction. Furthermore, such a model might give the industry a tool to calculate the effects of certain changes in diet composition, birds or management on financial returns.

The metabolism of non-essential amino acids might be an interesting entrance for further improvement of nutritive knowledge. It is rather peculiar to see that at low dietary NEAA levels apparently growth is impaired while still approximately 25 % of absorbed nitrogen is excreted as uric acid and thus must have passed the stage of non-essential amino acids. The reason for this discrepancy or paradox should in my opinion be the major subject for future research. Experiments should start with a description, qualitative and quantitative, of the flows of these amino acids through the body. Secondly, the separate processes leading to loss of nitrogen

should be studied. For instance, how is uric acid synthesis stimulated, is it triggered by a level of an amino acid, NH_3 or is it hormonal or neural regulated ? If this information is available it might be possible to delay or decrease uric acid synthesis and thus increase availability of NEAA for protein synthesis. This could result in an important improvement of N-efficiency.

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SUMMARY

The present thesis describes the development of a physiological feeding strategy for protein in broilers. This project was initiated to improve the efficiency of deposition of dietary nitrogen into animal product in order to reduce nitrogen excretion to the environment. The physiological feeding strategy was based on the consideration that feeding the birds according to their requirements based on physiological status would result in the highest efficiency. At the start of the project, requirement data for amino acids were considered not adequate. For this reason the amino acid pattern required was estimated based on a factorial approach. Generally in a factorial approach requirements are divided in a requirement for production and a requirement for non-productive purposes. The required amino acid pattern for production was based on the amino acid pattern of body accretion. Due to the high growth rate of broiler chicks and high N-efficiency the largest part of dietary amino acids is used for nett protein deposition. This implies that the required dietary amino acid pattern is mainly determined by the amino acid pattern of body accretion.

In the first chapter whole body amino acid composition of broiler chicks was studied with the aim to provide this basis amino acid pattern for diet formulation. Three genotypes were studied; a commercial broiler hybrid (Com) and two experimental lines: one selected for high growth rate (GL) and one selected for efficient feed conversion (FC). Male and female Com chicks were compared. Change of whole body amino acid composition with age was studied in male Com chicks by weekly analysis.
Whole body amino acid composition at the consecutive sampling moments showed several small, but statistically significant ($P<0.001$), differences. The differences in the amino acid pattern of body accretion in 0-21 and 22-49 days

of age leads to the proposal to compose different dietary formulations in these age-periods.
The three genotypes differed in relative amino acid composition. Analysis of growth pattern revealed no differences between Com and GL genotype, the FC genotype however had a clearly different growth pattern. Similar growth patterns did not result in similar whole body amino acid composition.
Compared to male Com birds, whole body of female Com birds contained relative less essential amino acids (cys, met, val, tyr, phe and his) and more non-essential (glu, asp) amino acids. It is suggested that the amino acid pattern of whole body accretion in male Com chicks can be used as a basis for diet formulation for broiler chicks of different genotypes and sexes.

When formulating diets based on the required amino acid pattern it is essential to know the nett availability of nutrients from the diet. For this reason in chapter 2 a digestibility study is included in this thesis. The influences of genotype, age and sex on droppings digestibility coefficients of a compound food were studied using male and female broiler chickens of three different genotypes at 2, 4 and 6 weeks of age. Because the traditional method of determination of droppings digestibility coefficients of nitrogen may lead to systematic errors in estimating the feeding value of foodstuffs, a method is proposed to determine the ileal digestibility coefficients. The ileal method is compared with the droppings method for a mixed food and for two foodstuffs: wheat and solvent-extracted soyabean meal.
Birds selected for efficient food conversion showed distinctly higher digestibility coefficients for all nutrients than birds selected for high growth potential or birds from a commercial strain. The influence of age on digestibility coefficients was not consistent. Female birds showed digestibility coefficients which were, in general, 3% higher than those of male chickens. Interactions of genotype and sex and between genotype and age for energy metabolisability were the only interactions observed for digestibility measurements.
The method of determination influenced the amino acid digestibility coefficients of the mixed food and the relative feeding values of wheat and soyabean meal.
It is important to use well defined animals (genotype, sex, age) in evaluating foodstuffs. The preferred method for determination of digestibility coefficients of nitrogen and amino acids is based on ileal sampling, although the differences in amino acid digestibility coefficients were small between methods.

After amino acids are made available from the diet, the metabolic utilisation of the amino acids determines whether they are used for either protein synthesis or for non-productive purposes. The utilisation of amino acids for non-productive purposes was studied with $^{14}CO_2$ breath test measurements. In these measurements a tracer quantitiy of a [^{14}C]amino acid is injected in a bird with a defined physiological status and exhaled $^{14}CO_2$ is collected during 4½ hours after injection. $^{14}CO_2$ recovery is a measure for oxidation of the amino acid.

In chapter 3 the effect of nutritional status (dietary lysine content, time of injection relative to the meal) on $^{14}CO_2$ recovery from intraperitoneal (ip) injected [U-^{14}C]lysine was studied in broiler chicks.

It was found that ip injection of [^{14}C]labelled amino acids ([U-^{14}C]lysine) sometimes resulted in unrealistic high recovery values in $^{14}CO_2$ breath test measurements. The most probable explanation is that with ip-injection in some cases label is injected in an abdominal air sac. In this case part of the substrate is not converted to $^{14}CO_2$ but is straight excreted as an aerosol. For this reason ip-injection of tracer amino acids is considered to be risky in chickens and therefore subcutaneous injection is used in further experiments.

When only the realistic, lower, range of $^{14}CO_2$ recoveries from [U-^{14}C]lysine were considered, lysine content of the diet caused a response in $^{14}CO_2$ recovery while time of injection did not give a detectable effect. Low lysine diet resulted in lower $^{14}CO_2$ recovery, indicating a restriction of lysine oxidation.

In birds conditioned on a lighting regime of 1L:2D, $^{14}CO_2$ recovery from subcutaneous injected [1-^{14}C]leucine or [1-^{14}C]valine was studied within one cycle of the lighting regime with injections at 30, 60, 120 or 180 minutes after onset of a half-hour meal.

For both [1-^{14}C]leucine and [1-^{14}C]valine $^{14}CO_2$ recovery was higher when injection at 30 or 60 min after onset of a half hour meal is compared to injection at 120 or 180 min. This can be interpreted as that a $^{14}CO_2$ breath test measurement should be started at 30 or 60 minutes after onset of a meal when amino acid utilisation in fed animals is to be investigated.

In chapter 4 the effect of dietary amino acid content and of length of adaptation period on $^{14}CO_2$ recovery from [^{14}C]amino acids was studied in broiler chick breath test measurements.

A lower (70% of normal) dietary lysine level increased $^{14}CO_2$ recovery from [1-^{14}C]leucine as well as from [1-^{14}C]valine during the first growth phase (5-22 d of age). In older chicks this effect was less pronounced. A reduced vs increased dietary methionine level (67 vs 133 % of normal) increased $^{14}CO_2$ recovery from

[1-^{14}C]leucine only at about 4 weeks of age. At a low dietary lysine level $^{14}CO_2$ recovery from [U-^{14}C]lysine was lower than at a normal lysine level. Length of adaptation period to a low lysine diet (1, 2, 3 or 5 d) had no statistically significant effect on $^{14}CO_2$ recovery from [1-^{14}C]leucine.
The utilization of leucine and valine for protein synthesis, as indicated by the complement of the $^{14}CO_2$ recovery from [^{14}C]amino acids, is affected by dietary limiting amino acid level. The age-dependent effect of dietary methionine level suggests that the method is especially suited for assessment of specific requirements during specific stages of development. The absence of influence of length of adaptation period to a low lysine diet on $^{14}CO_2$ recovery from [^{14}C]leucine suggests that adaptation is either rapid (< 1 day) or not measured with this approach. It is concluded that $^{14}CO_2$ recovery from [^{14}C]amino acids indicates amino acid adequacy of the diet fed immediately preceeding (20h period) the breath test measurement.

In chapter 5, nitrogen efficiency was studied in birds fed diets based on the amino acid pattern of body accretion at three levels of protein/energy (P/E) ratio, supplemented with Met, Arg, Glu and Gly, individually, as a group or not. This study was performed to see whether the amino acid pattern of body accretion could be used as a basis for diet formulation or that specific alterations in the pattern were required. The level of protein/energy (P/E) ratio may affect N-efficiency. Furthermore, there may be an interaction between dietary composition and genotype. Growth performance and nitrogen efficiency were measured in male and female chicks of two different genotypes, fed diets differing in amino acid composition and in P/E ratio. There were twelve diets. Three diets, of which the amino acid pattern was similar to the amino acid pattern of body accretion, differed only in P/E ratio (High: 17.8 and 19.3 g CP/MJ AME during 0-21 d of age and 22 d-slaughter age, respectively; Medium and Low: 94 % and 87 % of High). Six other diets were based on the medium P/E ratio but were supplemented with either Met, Arg, Glu, Gly, Glu + Gly or all four amino acids together. Two more diets were based on the low or high P/E ratio and supplemented with all four amino acids. Diet 12 was a control diet with a semi-practical composition. Male chicks of a commercial broiler hybrid (Com) were fed all diets. Male chicks from a population selected for low feed conversion ratio (FC) were fed the three diets differing in P/E ratio plus the medium P/E diet supplemented with all four amino acids. This latter diet and the unsupplemented medium P/E diet were fed to female chicks of both genotypes.

It was shown that no amino acid supplementation improved N-efficiency; Met-supplementation improved growth rate at similar N-efficiency while other supplementations negatively affected N-efficiency. Both genotypes reacted similar to differences in dietary composition. This supports the hypothesis that the amino acid pattern of body accretion is a good reference for dietary amino acid pattern. Female chicks also reacted similar to differences in dietary composition as male chicks but attained a much lower N-efficiency. Growth performance was lower at low P/E ratio while N-efficiency was lower at high P/E ratio. This shows that maximal growth performance and maximal N-efficiency can not be attained simultaneously. It is calculated that, compared to male Com chicks fed the control diet, feeding the diet with highest N-efficiency (low P/E ratio) will result in a reduction of N-excretion of 11 %. When this diet is fed to male FC chicks, N-excretion will be only 67 % of that of male Com chicks fed the control diet. It is concluded that dietary as well as genetic improvement will result in lower N-excretion.

Based on the previous studies it was hypothesized that nitrogen efficiency could be further increased by a decrease in dietary supply of specific non-essential amino acids (Glu + Asp + Ala, NEAA) since these amino acids are synthesized as a result of the degradation of essential amino acids. Normally these amino acids function to transport amino acid nitrogen from the sites of amino acid degradation to the site of where they are combined with glycine to synthesize uric acid which is subsequently excreted. If the excretion of uric acid is increased by extra dietary non-essential amino acid nitrogen this will result in a extra demand for glycine since glycine, contrary to the other amino acids contributing nitrogen to uric acid, is completely incorporated. Since glycine can be synthesized from threonine an increase in dietary NEAA level might result in an increased non-productive threonine requirement.

In chapters 6 and 7 three experiments are described in which responses of birds fed several different diets are compared. Chapter 6 describes technical performance such as growth rate, feed conversion ratio (FCR) and nitrogen efficiency whereas chapter 7 describes weight and yield percentages of edible and other portions after slaughter. In the first experiment described in chapters 6 and 7 a comparison is made between a practical diet and experimental diets based on either data from the literature or the amino acid pattern of body accretion plus some adaptations of both these patterns. It was shown that the amino acid pattern of body accretion can be used as basis for calculation of

amino acid requirements. There were no differences in response between a diet based on literature or a diet based on the amino acid pattern of body accretion. The practical diet resulted in a lower N-efficiency, higher growth rate and lower FCR. In the second growth phase (15-35 d of age) there was a positive effect of extra methionine (relative to the pattern of body accretion) on growth and FCR without negative effect on N-efficiency. This implies that in this growth phase dietary methionine level should be higher than that based on the level of body accretion.

It was shown that absolute meat production was only slightly affected by dietary amino acid pattern while for relative yields several significant but minor differences were found. Breast meat percentage was highest in birds fed a diet with extra Met and lowest in birds fed a diet with extra Glu. It is concluded that within three proposed dietary amino acid patterns variation in dietary amino acid pattern had only minor influence on slaughter efficiency.

In the second experiment described in chapters 6 and 7 it was tested whether it was possible to promote utilisation of non-essential amino acids formed in the obligatory breakdown of essential amino acids for nett protein synthesis by lowering the dietary non-essential amino acid level. Nine diets with varying levels of glutamic acid + aspartic acid + alanine (NEAA) were fed to male broiler chicks. It was shown that with lower NEAA levels growth rate was lowered, FCR was higher, but N-efficiency was higher. Maximal growth rate and maximal N-efficiency can thus not be reached simultaneously.

It was shown that variation in dietary NEAA level had no effect on yields of body parts as percentage of live weight. Total production was related to live weight and was decreased with decreasing dietary NEAA level.

The third experiment described in chapters 6 and 7 was based on the theory that high NEAA excess will lead to extra uric acid production which will lead to an increase in glycine requirement. Since glycine can be formed from threonine, threonine requirement might be affected by NEAA level. In an experiment with three threonine and three NEAA levels it was shown that at high NEAA level the effect of low threonine level was more severe than at low NEAA level. A distinct interaction between dietary Thr and NEAA level on yields was observed. This supports the hypothesis that the requirement of threonine is determined not only by the requirement for body accretion but also by a requirement for glycine production to excrete excess nitrogen.

In general it can be concluded that the developed physiological feeding strategy can improve nitrogen efficiency. However, the limits to practical application may depend on an economic evaluation. Moreover, an improved knowledge concerning utilisation of non-essential amino acids for uric acid and protein synthesis may result in further improvement in the physiological feeding strategy.

SAMENVATTING

Dit proefschrift beschrijft de ontwikkeling van een fysiologische voedingsstrategie voor eiwit bij vleeskuikens. Dit project is gestart met het doel om de efficiëntie waarmee eiwit in het voer wordt omgezet in eiwit in het dierlijk produkt te verhogen om op deze wijze de stikstofuitstoot naar het milieu te verlagen. Deze fysiologische voedingsstrategie is gebaseerd op de gedachte dat de hoogste efficiëntie bereikt kan worden indien de voeding op ieder moment precies voorziet in de fysiologische behoefte van dat moment. Omdat bij aanvang van het project onvoldoende gegevens beschikbaar waren met betrekking tot de behoefte aan aminozuren is het aminozuurpatroon van de behoefte geschat op basis van een factoriële benadering. Over het algemeen wordt bij een factoriële benadering de behoefte opgedeeld in enerzijds een behoefte voor produktie en anderzijds een behoefte voor niet-produktie doeleinden. Het benodigde aminozuurpatroon voor produktie is gebaseerd op het aminozuurpatroon van de groei (lichaamsaanzet). Omdat vleeskuikens een hoge groeisnelheid en een hoog N-rendement (stikstofaanzet gedeeld door stikstofopname) hebben wordt het grootste deel van de aminozuren in het voer gebruikt voor netto eiwit-synthese. Dit betekent dat het aminozuurpatroon van de behoefte vooral bepaald wordt door het aminozuurpatroon van de lichaamsaanzet.

In het eerste hoofdstuk wordt het aminozuurpatroon van het gehele lichaam van vleeskuikens bestudeerd om als basis te dienen voor het samenstellen van voeders. Er zijn drie genotypen bestudeerd; een commercieel vleeskuiken (Com) en twee experimentele genotypen: een die geselecteerd was op hoge groeisnelheid (GL) en een die geselecteerd was op een lage voederconversie (FC). Van het Com genotype zijn haan- en henkuikens vergeleken. De

verandering van het aminozuurpatroon met de leeftijd is gevolgd voor Com haankuikens door wekelijks het aminozuurpatroon in het gehele dier te bepalen. Het aminozuurpatroon van het gehele dier op de opeenvolgende meetmomenten vertoonde verschillende kleine doch statistisch significante ($P<0.001$) verschillen. De verschillen in aminozuurpatroon van de lichaamsaanzet tussen de perioden 0-21 en 22-49 dagen leeftijd leidde tot het voorstel om voor deze perioden verschillende voersamenstellingen te maken.
Het aminozuurpatroon in het gehele dier van de drie genotypen verschilde duidelijk. Er was geen verschil in het groeipatroon van Com en GL dieren terwijl de FC dieren een duidelijk verschillend groeipatroon hadden. Deze gelijke groeipatronen resulteerden echter niet in een gelijk aminozuurpatroon in het dier. Vergeleken met Com haankuikens bevatte het lichaam van Com henkuikens minder essentiële aminozuren (Cys, Met, Val, Tyr, Phe en His) en meer niet-essentiële (Glu, Asp) aminozuren. Voorgesteld wordt het aminozuurpatroon van de lichaamsaanzet van Com haankuikens te gebruiken als basis voor het samenstellen van voeders voor vleeskuikens, inclusief henkuikens en kuikens van andere genotypen.

Als voeders samengesteld worden op basis van het aminozuurpatroon van de behoefte is het essentieel om de netto beschikbaarheid van nutrienten uit het voer te weten. Daarom is in hoofdstuk twee van dit proefschrift een verteringsstudie opgenomen. Om de invloed van genotype, leeftijd en geslacht op de verteerbaarheid, op faecaal niveau, van een compleet voer te bestuderen zijn voor haan- en henkuikens van drie genotypen op 2, 4 en 6 weken leeftijd verteringscoëfficiënten bepaald. Omdat bij de traditionele bepaling van de verteerbaarheid van stikstof op faecaal niveau sytematische fouten in de waardering van voedermiddelen op kunnen treden is een methode voorgesteld om de ileale verteerbaarheid te bepalen. Voor het complete voer en twee grondstoffen (tarwe en sojaschroot) is deze ileale methode vergeleken met de methode op faecaal niveau.
Kuikens van het FC genotype vertoonden duidelijk hogere verteringscoëfficiënten voor alle nutrienten in vergelijking tot Com of GL kuikens. De invloed van leeftijd op de verteerbaarheid was niet consequent. De verteringscoëfficiënten bepaald bij henkuikens waren, over het algemeen, 3 % hoger dan die bij haankuikens. Alleen voor omzetbaarheid van de energie zijn er interacties tussen genotype en geslacht en tussen genotype en leeftijd gevonden.

De bepalingsmethode had invloed op de bepaalde verteringscoëfficiënten van het complete voer en op de relatieve voederwaarde van tarwe en sojaschroot.
Bij waardering van grondstoffen is het belanrijk om de te gebruiken dieren goed te definiëren voor wat betreft genotype, leeftijd en geslacht. Ondanks dat de verschillen in verteringscoëfficiënten tussen de faecale en ileale methode klein waren wordt voor de bepaling van verteringscoëfficiënten van stikstof en aminozuren de ileale methode geprefereerd.

Als aminozuren beschikbaar zijn uit het rantsoen bepaalt het metabolisme of deze aminozuren gebruikt worden voor eiwitsynthese of voor niet-produktie doeleinden. Dit gebruik voor niet-produktiedoeleinden is bestudeerd met behulp van $^{14}CO_2$ ademanalyse. Hierbij wordt bij een kuiken met een bepaalde gedefiniëerde status een tracer dosis van een [^{14}C]aminozuur geïnjecteerd waarna gedurende 4½ uur de uitgeademde $^{14}CO_2$ wordt opgevangen. Deze $^{14}CO_2$ recovery is een maat voor de oxidatie van het aminozuur.
In hoofdstuk 3 is bij vleeskuikens het effect van nutritionele status (lysinegehalte van het voer, tijdstip van injectie ten opzichte van de maaltijd) op de $^{14}CO_2$ recovery van intra-peritoneaal geïnjecteerd [U-^{14}C]lysine onderzocht.
Het bleek dat intra-peritoneale injectie van [^{14}C]aminozuur ([U-^{14}C]lysine) soms leidde tot onrealistisch hoge $^{14}CO_2$ recovery bij ademanalyse. De meest waarschijnlijke verklaring hiervoor is dat bij ip-injectie in sommige gevallen het label in een abdominale luchtzak geïnjecteerd werd. In dat geval is het substraat niet omgezet in $^{14}CO_2$ maar direct, als aerosol, uitgeademd. Dit is de reden dat ip-injectie van tracer aminozuren bij kippen riskant is. Daarom is bij verdere experimenten subcutaan geïnjecteerd.
Als alleen de reële, lage, waarden van de $^{14}CO_2$ recovery van [U-^{14}C]lysine bekeken werden bleek dat het lysinegehalte van het voer wel een respons te zien gaf, terwijl tijdstip van injectie geen effect had. Het laag lysine rantsoen resulteerde in een lage $^{14}CO_2$ recovery, wat een beperking in lysine oxidatie aangeeft.
Het effect van tijdstip van injectie binnen een lichtcyclus ten opzichte van een maaltijd is bestudeerd bij dieren die gewend waren aan een lichtschema van 1 uur licht afgewisseld met 2 uur donker (1L:2D). De $^{14}CO_2$ recovery is bepaald na een subcutane injectie van [1-^{14}C]leucine of [1-^{14}C]valine op 30, 60, 120 of 180 minuten na aanvang van een half uur durende maaltijd.
Voor zowel [1-^{14}C]leucine als [1-^{14}C]valine bleek de $^{14}CO_2$ recovery hoger voor de injectietijdstippen 30 en 60 minuten na aanvang van de maaltijd in vergelijking met injectie op 120 of 180 minuten na aanvang van de maaltijd. Een

interpretatie hiervan is dat als het gebruik van aminozuren in het metabolisme van gevoede dieren bestudeerd moet worden de $^{14}CO_2$ ademanalyse 30 of 60 minuten na aanvang van de maaltijd gestart moet worden.

In hoofdstuk 4 is bekeken wat de invloed is van het aminozuurgehalte van het rantsoen en van de lengte van de periode van gewenning aan het rantsoen op de $^{14}CO_2$ recovery bij $^{14}CO_2$ ademanalyse bij vleeskuikens.
Tijdens de eerste groeifase (5-22 dagen) bleek een lager lysinegehalte (70 % van normaal) de $^{14}CO_2$ recovery van zowel [1-^{14}C]leucine als [1-^{14}C] valine te verhogen. Bij oudere kuikens was dit effect minder uitgesproken. Alleen bij vier weken oude kuikens bleek vergelijking van een verlaagd en een verhoogd methioninegehalte in het rantsoen (67 vs 133 % van normaal) een verschil in $^{14}CO_2$ recovery van [1-^{14}C]leucine op te leveren. Bij een laag lysinegehalte in het rantsoen was de $^{14}CO_2$ recovery van [U-^{14}C]lysine lager dan bij een normaal lysinegehalte. De lengte van de gewenningsperiode aan een laag lysine rantsoen (1, 2, 3 of 5 dagen) had geen statistisch significant effect op de $^{14}CO_2$ recovery van [1-^{14}C]leucine.
Het gebruik van leucine en valine voor eiwitsynthese, afgeleid uit het complement van de $^{14}CO_2$ recovery van [^{14}C]aminozuren, wordt beïnvloed door het aminozuurgehalte van het rantsoen. Het leeftijdsafhankelijke effect van het methioninegehalte suggereert dat deze methode vooral geschikt is om specifieke behoeften gedurende speficieke ontwikkelingsstadia te onderzoeken. De afwezigheid van een invloed van lengte van de gewenningsperiode aan een rantsoen met een laag lysinegehalte op de $^{14}CO_2$ recovery van [1-^{14}C]leucine suggereert dat of de aanpassing erg snel is (binnen een dag) of dat aanpassing niet op deze manier te meten is. De conclusie was dat de $^{14}CO_2$ recovery van [^{14}C]aminozuren aangeeft in hoeverre het aminozuurgehalte van het rantsoen opgenomen gedurende een periode van 20 uur direct voorafgaande aan de meting adequaat was.

In hoofdstuk 5 is het N-rendement bestudeerd van kuikens gevoerd met rantsoenen gebaseerd op het aminozuurpatroon van de lichaamsaanzet, op drie niveaus van eiwit/energie (P/E) verhouding en aangevuld met Met, Arg, Glu en Gly, afzonderlijk, als groep of niet aangevuld. Dit onderzoek is uitgevoerd om na te gaan of het aminozuurpatroon van de lichaamsaanzet bruikbaar was als basis voor rantsoensamenstelling of dat specifieke veranderingen in het patroon nodig waren. Het niveau van de P/E verhouding zou het N-rendement kunnen beïnvloeden. Verder zou er een interactie kunnen zijn tussen

rantsoensamenstelling en genotype. Daarom is groei en N-rendement bepaald bij haan- en henkuikens van twee genotypen, gevoed met rantsoenen verschillend in aminozuurpatroon en in P/E verhouding. Er waren in totaal 12 rantsoenen. Drie rantsoenen, waarvan het aminozuurpatroon gebaseerd was op het aminozuurpatroon van de lichaamsaanzet, verschilden alleen in P/E verhouding. De hoge P/E verhouding was 17.8 en 19.3 g RE/MJ OE gedurende respectievelijk de perioden 0-21 dagen en 22 dagen tot het moment van slachten. De midden en lage P/E verhouding waren 94 en 87 % hiervan. Zes andere rantsoenen waren gebaseerd op de midden P/E verhouding en aangevuld met Met, Arg, Glu, Gly, Glu + Gly of alle vier aminozuren gezamenlijk. Twee andere rantsoenen waren gebaseerd op het hoge of lage P/E rantsoen en aangevuld met alle vier aminozuren. Het twaalfde rantsoen was een controlerantsoen met een semi-praktijk samenstelling. Alle rantsoenen zijn verstrekt aan Com haankuikens terwijl haankuikens van het FC genotype alleen de drie rantsoenen met verschil in P/E verhouding plus het midden P/E rantsoen aangevuld met alle vier aminozuren kregen. Dit laatste rantsoen en het midden P/E rantsoen zonder aanvulling met aminozuren is ook aan henkuikens van beide genotypen verstrekt.

Het bleek dat geen enkele aanvulling met aminozuren het N-rendement verbeterde; extra Met verbeterde de groeisnelheid bij gelijkblijvend N-rendement terwijl iedere andere aanvulling het N-rendement negatief beïnvloedde. Beide genotype reageerden op soortgelijke wijze op verschillen in rantsoensamenstelling. Dit ondersteunt de hypothese dat het aminozuurpatroon van de lichaamsaanzet een goede basis is voor het aminozuurpatroon van het rantsoen. Henkuikens reageerden eveneens op soortgelijke wijze op verschillen in rantsoensamenstelling als haankuikens maar bereikten een veel lager N-rendement. De groeisnelheid was lager bij een lagere P/E verhouding terwijl het N-rendement lager was bij hogere P/E verhouding. Dit laat zien dat maximale groeiprestatie en maximaal N-rendement niet tegelijk bereikt kunnen worden. Het is berekend dat, vergeleken met Com haankuikens gevoerd met het controle rantsoen, gebruik van het rantsoen met het hoogste N-rendement (laag P/E rantsoen) zou resulteren in een vermindering van de N-uitstoot met 11 %. Als dit rantsoen aan haankuikens van het FC genotype gevoerd wordt zal de N-uitstoot slecht 67 % zijn van die van Com haankuikens gevoerd met het controle rantsoen. De conclusie was dat zowel verbetering in het rantsoen als genetische verbeteringen de N-uitstoot kunnen beperken.

Gebaseerd op de voorgaande studies is de hypothese opgesteld dat het N-rendement verder verhoogd zou kunnen worden door een verlaging van het aanbod aan niet-essentiële aminozuren (Glu + Asp + Ala, NEAA) in het rantsoen omdat deze aminozuren gesynthetiseerd worden bij de afbraak van essentiële aminozuren. De functie van deze aminozuren is het transporteren van aminozuurstikstof van de plaats van aminozuurafbraak naar de plaats van urinezuursynthese. Hier worden ze, samen met glycine gebruikt om urinezuur te synthetiseren dat vervolgens uitgescheiden wordt. Als de uitscheiding van urinezuur verhoogd wordt door een verhoogde opname van niet-essentiële aminozuurstikstof uit het rantsoen zal dit een extra glycinebehoefte tot gevolg hebben. Glycine wordt namelijk, in tegenstelling tot de andere aminozuren die stikstof leveren voor de urinezuursynthese, volledig in urinezuur ingebouwd. Omdat glycine uit threonine gemaakt kan worden zou een verhoging van het NEAA gehalte in het rantsoen een verhoogde, niet aan de produktie gerelateerde, threonine behoefte tot gevolg kunnen hebben.

In de hoofdstukken 6 en 7 worden drie experimenten beschreven waarin de respons van kuikens gevoed met een aantal verschillende rantsoenen vergeleken wordt. Hoofdstuk 6 beschrijft de technische prestaties zoals groeisnelheid, voederconversie (FCR) en N-rendement terwijl in hoofdstuk 7 de slachtgewichten en relatieve aandelen van eetbare en andere delen na het slachten beschreven worden. In het eerste experiment wordt een vergelijking gemaakt tussen een praktijkrantsoen en experimentele rantsoenen met een aminozuurpatroon gebaseerd op literatuurwaarden of op het aminozuurpatroon van de lichaamsaanzet plus enkele aanpassingen van deze beide patronen. Het bleek dat het aminozuurpatroon van de lichaamsaanzet gebruikt kon worden als basis voor de rantsoensamenstelling. Er waren geen verschillen in respons tussen dieren gevoerd met het rantsoen gebaseerd op de literatuurwaarden en het rantsoen gebaseerd op het aminozuurpatroon van de lichaamsaanzet. Het praktijkrantsoen resulteerde in een lager N-rendement, hogere groeisnelheid en een lager FCR. Een verhoogd methioninegehalte had in de tweede groeifase (15-35 dagen) een positief effect op groeisnelheid en FCR zonder het N-rendement negatief te beïnvloeden. Dit betekent dat in deze groeifase het methioninegehalte in het rantsoen hoger moet zijn dan overeenkomstig het aminozuurpatroon van de lichaamsaanzet.
Het bleek dat absolute vleesproduktie nauwelijks beïnvloed werd door het aminozuurpatroon van het rantsoen terwijl bij de relatieve aandelen verschillende statistisch significante doch geringe verschillen tussen rantsoenen gevonden

werden. Het percentage borstvlees (filet) in de kuikens was het hoogst bij het rantsoen met extra methionine en het laagst bij het rantsoen met extra glutaminezuur. De conclusie was dat binnen de gebruikte aminozuurpatronen verschil in aminozuurpatroon in het rantsoen slechts een geringe invloed had op relatieve aandelen van eetbare delen.
In het tweede experiment beschreven in hoofdstuk 6 en 7 is getest of het mogelijk was om het gebruik voor netto eiwitsynthese van niet-essentiële aminozuren, ontstaan bij de afbraak van essentiële aminozuren, te stimuleren door het gehalte aan niet-essentiële aminozuren in het rantsoen te verlagen. Negen rantsoenen waarin het gehalte aan glutaminezuur, asparaginezuur en alanine (NEAA) was gevarieerd werden verstrekt aan mannelijke vleeskuikens. Het bleek dat bij een lager NEAA gehalte de groeisnelheid verlaagd, de FCR verhoogd maar het N-rendement verhoogd was. Maximale groeisnelheid en maximaal N-rendement gaan dus niet samen.
Het bleek dat variatie in het NEAA gehalte in het rantsoen geen effect had op het aandeel van verschillende lichaamsdelen in het levend gewicht. De totale produktie van eetbare delen was gerelateerd aan het levend gewicht en daalde bij verlaging van het NEAA gehalte in het rantsoen.
Het derde experiment dat in hoofdstuk 6 en 7 beschreven wordt is gebaseerd op de theorie dat een hoog overschot aan NEAA in het rantsoen de urinezuurproduktie zal verhogen waardoor de glycine behoefte verhoogd wordt. Omdat glycine uit threonine gevormd kan worden zou de threonine behoefte beïnvloed kunnen worden door het NEAA gehalte van het rantsoen. In een experiment met drie NEAA en drie Thr niveaus werd aangetoond dat bij een hoog NEAA gehalte het effect van een laag Thr gehalte ernstiger was dan bij een laag NEAA gehalte. Er was een duidelijke interactie tussen NEAA en Thr gehalte in het rantsoen voor aandeel van eetbare delen ten opzichte van het lichaamsgewicht. Dit ondersteunt de hypothese dat de threonine behoefte niet alleen bepaald wordt door de behoefte voor lichaamsaanzet maar ook door de behoefte voor produktie van glycine ten behoeve van excretie van stikstof.

Een algemene conclusie is dat de ontwikkelde voedingsstrategie het N-rendement kan verbeteren. Echter, de grenzen bij praktische toepassing zijn afhankelijk van een economische evaluatie. Bovendien kan een verbeterde kennis van het gebruik van niet-essentiële aminozuren voor urinezuur- en eiwitsynthese de fysiologische voedingsstrategie verder verbeteren.

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Als ik naar het bovenstaande kijk vraag ik me ten eerste af wie ik allemaal niet genoemd heb (dat zijn er zeker een stuk of twintig) en ten tweede of ik zelf nog wel iets uitgevoerd heb. Nou ja, dat zal ik allemaal wel horen op 20 juni....

CURRICULUM VITAE

Robert Antonius Hermanus Maria (Rob) ten Doeschate werd op 25 november 1965 geboren in Nijmegen. Hij groeide op op een boerderij in het dorp Puiflijk. In 1984 behaalde hij het VWO-diploma aan het Pax Christi College in Druten waarna hij in Wageningen begon aan de studie Zoötechniek. In 1986 heeft hij de studie gedurende een half jaar onderbroken om in het bestuur van Katholieke Studenten Vereniging Sint Franciscus Xaverius de functie van commissaris mensa te vervullen. Hierna is de studie vervolgd en afgesloten in maart 1990. Het eerste afstudeervak was een studie naar het eiwit- en energiemetabolisme van slachtkuikens, bij de vakgroep Dierfysiologie. De stagetijd heeft hij doorgebracht bij de mengvoederfirma Koopmans BV in Leeuwarden. Hierna volgde een afstudeervak veevoeding met als onderwerp de opname van hooi en silage bij ooien, uitgevoerd bij het IVVO in Lelystad. Op 15 maart 1990 was hij werkzaam als Assistent in Opleiding (AIO) bij de vakgroep Fysiologie van Mens en Dier binnen het project 'Het ontwikkelen van een Fysiologische Voedingsstrategie ter beperking van de N-uitstoot vanuit de dierlijke produktie. Voorbeeldstudie bij slachtkuikens'. Gedurende de eerste anderhalf jaar en in de eerste helft van 1994 was hij gedetacheerd bij COVP het Spelderholt in Beekbergen waarmee samengewerkt werd in het project. Op 15 december 1994 is zijn AIO-contract beëindigd.