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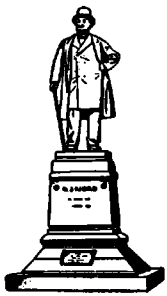
A study of certain factors influencing protein utilization in rats and pigs

By

Bjørn O. Eggum



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Bjørn O. Eggum

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CHAPTER I

Introduction

The primary aim of the present work has been to develop an experimental technique permitting the use of rats in studies of physiological aspects of animal nutrition and providing results normative for the nutrition of domestic animals. Rats have an advantage compared to several of our domestic animal species in that they can be induced to consume a feedstuff of restricted composition over considerable periods. Thus trials can be carried out with isolated feedstuffs, thereby eliminating possible complementary effects. This must necessarily give rise to increased experimental accuracy in such trials.

The present work is chiefly concerned with the quality of the protein in our feedstuffs and the majority of the biological data have been obtained in trials with rats. At the Institute of Animal Science protein quality is traditionally evaluated on the basis of nitrogen balances and this tradition has been followed in the present experiments. The method of evaluation employed is that of *Mitchell* (1924a) which gives the biological values of the protein compounds; this method is widely used and is accepted internationally. The method also has the advantage of including the true digestibility of the protein as well as the biological value. As for all other biological methods, this method of evaluation of protein quality also suffers from certain deficiencies; these are discussed in this report.

A number of experimental factors influencing the results obtained are considered. These investigations have emphasized the necessity of working under strictly standardized conditions when employing the *Mitchell* method.

It should be noted at this stage that the biological value of a protein fed as the sole protein source provides little information on the supplementary value of such a protein in a mixed diet. The biological value of a mixture of two protein sources will be higher than the individual values of the two components when limited by different amino acids. This concept forms the basis of the phenomenon of mutual supplementation between proteins. For such measurements biological value is an extremely sensitive criterion.

A protein does not contain amino acids as such, but only the condensed units linked in peptide chains. Digestion in the gastro-intestinal tract proceeds by the hydrolysis of these peptide links to form free amino acids. However, a certain proportion of the total amino acids in the dietary constituents may be biologically unavailable. The nutritive value of a protein is known to depend not only on the pattern of the component amino acids but also on their biological

availability and on the fact that the levels of amino acids present in a feed are not necessarily the levels available to the body.

Availability of amino acids can be reduced by incomplete digestion and absorption, by the presence of inhibitors of digestive enzymes, or by heat damage to feed proteins and amino acids. Thus the amino acid constituents of different feedstuffs are not necessarily additive and consequently a linear optimalisation of the amino acid content of feed mixtures would be difficult to accomplish simultaneously with an optimalisation of the biological value of the mixture.

Several methods are found which attempt to elucidate the availability of amino acids, but all appear to be subject to varying degrees of inaccuracy. In the present experiments attempts have also been made to measure amino acid availability. In these experiments it was considered appropriate to employ the faecal analysis method of *Kuiken & Lyman* (1948). This method is based on a calculation of dietary amino acids and amino acids excreted with the faeces, but has been criticized by many workers, chiefly on the basis of the microbial activity in the digestive tract. This problem is, however, discussed in this report and has also been examined experimentally.

Since the majority of the present investigations are performed with rats, it is of interest to consider whether the results obtained are also applicable to domestic animals. In order to study this aspect investigations of protein quality have been carried out in 15 different protein sources using both rats and pigs, thereby enabling a direct comparison of these two species. Since these comparisons confirmed the value of rats in this connection, a number of additional experiments were carried out with rats and the results obtained are considered applicable to livestock nutrition in general.

It is difficult today for the research worker in any one branch of protein metabolism to comprehend the advances taking place on all fronts of the subject. Thus much relevant and valuable work might well have been overlooked in the present discussion. I would therefore ask authors of such work to excuse the unintentional omission of their contribution.

The lack of high quality food and feed proteins in large areas of the world is one of the great challenges of our generation and has prompted an intensive search for new protein sources. However, a thorough knowledge of the protein already available would help to alleviate this crisis and it is my hope that the present work may provide a small contribution in this direction.

CHAPTER II

The biological value as a measure of protein quality

A. General discussion

One of the most useful measurements involving nitrogen balance has been the determination of the biological value. The concept »biological value« as a measure of protein quality was introduced by *Thomas* (1909) and has since been taken up by many investigators. *Mitchell* and associates have done more than any other group of workers to render the determination of this value quantitative and meaningful (*Mitchell* 1929, 1944, 1954, 1959). The method is therefore generally referred to either as the *Thomas-Mitchell* method or as the *Mitchell* method.

The biological value of a dietary protein was defined by *Thomas* (1909) as the fraction of absorbed nitrogen retained in the body for maintenance and growth. The calculation of biological value (BV) therefore requires an estimation of the amount of nitrogen absorbed into the body and the amount of the absorbed nitrogen which is retained. *Mitchell* (1924a) described the method very concisely in the following words: »The method is based upon nitrogen balance data obtained under definite experimental conditions, and involves direct determinations of the amount of nitrogen in the feces and in the urine and indirect determinations of the fractions of the fecal nitrogen and of the urinary nitrogen that were of dietary origin. The biological value of the protein is taken as the percentage of the absorbed nitrogen (nitrogen intake minus fecal nitrogen of dietary origin) that is not eliminated in the urine«. This definition can be summarized by the following equation:

Equation 1:

$$BV = \frac{\text{N intake} - (\text{faecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogenous N})}{\text{N intake} - (\text{faecal N} - \text{metabolic N})} \times 100$$

In the numerator the faecal losses subtracted from the total intake are limited to the part actually undigested and the urinary loss is reduced by its endogenous fraction before being subtracted. The numerator therefore represents the total nitrogen utilized, including both that used in maintenance and that incorporated into growing tissues. Since the metabolic nitrogen is

also subtracted from the total faecal output in the denominator, the biological value computed is the percentage of digested nitrogen that is actually utilized. In excluding the metabolic and endogenous nitrogen from the losses, the *Thomas-Mitchell* method provides a measure of the efficiency of the absorbed protein for the combined functions of growth and maintenance.

The critical requirement in the *Mitchell* method is the provision of adequate methods for the estimation of metabolic N and endogenous N. *Mitchell* (1924a) first chose to estimate both quantities from data obtained when the experimental animals were given a protein-free diet. This procedure was later modified and metabolic and endogenous N were estimated from data obtained when rats were given a diet containing whole egg protein at a low concentration (*Mitchell & Carman* 1926). It was assumed that the egg protein was completely digested and utilized by the growing rat so that faecal and urinary nitrogen excretion represented unavoidable metabolic and endogenous losses. The metabolic nitrogen in the faeces was related to the intake of dry feed and the endogenous urinary nitrogen either by the body weight (*Mitchell* 1924a) or by a logarithmic function of the body weight (*Smuts* 1935, *Ashworth* 1935).

BV was found to be independent of the amount of feed eaten, but decreased when the protein content of the diet was increased (*Mitchell* 1924b). *Martin & Robinson* 1922), however, reported that BV was independent of the amount of protein eaten while in later work it was implied that within a certain range BV was independent of the protein content of the diet (*Armstrong & Mitchell* 1955, *Mitchell* 1955).

Njaa (1963), in a comprehensive study of the *Mitchell* method, verified the criticism mentioned above. He also discussed various other objections to this method. His work suggested faecal nitrogen excretion to be influenced by body weight independent of the intakes of feed and nitrogen. The urinary nitrogen excretion was studied in relation to the body weight and the growth rate of the rats and to their intakes of feed and nitrogen. The results indicated that the growth rate was of greater importance than the body weight for the variation in the excretion of urinary nitrogen. *Njaa* (1963) assumed that the body weight influenced only the level of endogenous nitrogen, whereas growth rate influenced both the exogenous and the endogenous nitrogen levels. Due to the large number of factors determining BV values, *Njaa* has stressed the necessity of strictly standardized conditions when the method of *Mitchell* is used in evaluating the proteins.

The analytical determination of the essential amino acid content in protein foods and feeds is logically the first step in protein evaluation. Such determinations permit the calculation of chemical scores (*Mitchell & Block* 1946) indicating the limiting amino acids, together with a prediction of the possible value of the protein in various dietary combinations.

Mitchell & Block (1946) and *Block & Mitchell* (1946–47) compared the amino acid content of a large number of foodstuffs containing whole egg and suggested that an approximate estimation of the BV of a protein (y) can be obtained from the maximum percentage deficit of the most limiting amino acid (x) by the equation $y = 100 - 0.634x$. However, calculation of nutritive values from amino acid patterns assumes that the pattern of amino acids absorbed into the body will be represented by the chemical analysis of the food. This assumption is not always correct since liberation of amino acids in the intestinal tract during digestion and differential rates of absorption may alter the pattern obtained from analytical data.

The validity of amino acid analyses for determining the nutritive value of protein is dependent on the biological availability of the amino acids. This aspect will be discussed in a later section.

B. Amino acid requirement for maintenance contra growth

The discussion of amino acid requirements and nutritive values of dietary proteins has indicated that the pattern of amino acids required for growth may differ from that required for maintenance and that the optimum patterns may vary with the physiological state of the individual. In general, however, data suggest that the over-all pattern optimum for growth is also the most suitable for maintenance and the repletion of depleted tissues (*Mitchell* 1959). For this reason egg proteins, having the highest nutritive value for growth, have been proposed as an ideal protein with which to obtain a reference pattern of essential amino acids. However, *Bender* (1961) found that the essential amino acids of defatted egg could be diluted 15% by weight with a mixture of dispensable amino acids without altering the nutritive value of the egg protein in the rat. However, further dilution with 30% of the dispensable amino acid mixture reduced the nutritive value.

Mitchell & Beadles (1950) demonstrated that relatively small amounts of lysine are required for maintenance in the rat compared with the amount needed for growth and hence the biological value for wheat gluten was relatively high for maintenance and low for growth. *Fisher et al.* (1960) stated that the differential response at two protein levels can be explained in terms of the relatively greater methionine requirement for maintenance at the low protein level compared to the relatively higher lysine to methionine ratio required for rapid growth at the high protein level.

The results of *McLaughlan & Noel* (1970) confirm previous suggestions that for lysine-deficient proteins no fixed relationship exists between protein quality determined at maintenance level and at levels supporting growth. They therefore concluded that methods such as net protein utilization and

biological value, which are estimated at relatively low protein levels, may be expected to give erroneously high results for foods in which lysine is the limiting amino acid.

Nevertheless, the biological value ot net protein utilization (*Block & Mitchell* 1946–47) is generally regarded as a specific characteristic of a food or feed protein as can be seen, for example, in Protein Requirements, FAO Nutritional Studies No. 16 1957 and Nutritional Data, H. J. Heinz Co. 1959. In addition new methods for protein evaluation are frequently standardized against values for BV or NPU (*Finlayson & Baumann* 1956, *Sheffner et al.* 1956, *Münchow & Bergner* 1968, *Eggum* 1970a, *Rølle & Eggum* 1971).

C. The significance of labile protein reserve in BV trials

The question of whether the presence of labile protein in the body is of advantage in acting as a readily available source of amino acids has been the subject of considerable discussion. *Holt et al.* (1962) did not consider rats fed a protein-free diet to have derived any advantage from a previous high intake of protein. *Samuels et al.* (1948) arrived at a similar conclusion in studies of the effect of the preceding diet on the survival of starving rats. *Shapiro & Fisher* (1962), however, suggested that reserve protein deposited in the chick by feeding a high intake of protein can induce better growth during a subsequent period of low protein intake.

It is generally recognized (*Allison* 1964) that the body does not store protein or create reserves in the same way as in fat metabolism. There is evidence, however, that certain tissue protein can be reversibly depleted and repleted by fluctuations in the quantity and quality of dietary proteins.

These tissue proteins can contribute to the free amino acid supply of the body, sometimes called the amino acid metabolic pool, and thereby help maintain essential structures and functions involving amino acids when the intake is reduced. These labile reserves represent only a small fraction of the total body protein, but the discussion concerning the significance and the role of protein reserves is often restricted to these very labile tissue proteins (*Allison et al.* 1963).

However, *Young* (1970) states in a review article that the pool of free amino acids in the muscle of large animals can represent an appreciable part of their daily requirements. Thus the pool of free threonine in muscle is equivalent to the threonine requirement of adult man for about 5 days. In young animals, however, the free amino acid pool of muscle provides a smaller proportion of the daily requirement of essential amino acids. This suggests that skeletal muscle may contribute a greater buffering effect on amino acid requirements in the adult than in the young human subject. The presence

of such pools of free amino acids may be of significance in determining the length of time which may elapse between the consumption of different amino acids and still allow these to be utilized i protein synthesis.

The question of delayed supplementation of amino acids has been reviewed by *Munro* (1970a) and it can be concluded that tryptophan is generally agreed to require simultaneous administration along with the other essential amino acids in order to be utilized for protein synthesis, whereas lysine can probably be fed several hours before or after feeding the other amino acids of the diet and still allow full utilization of the amino acid mixture. This would be compatible with the results of *Young* (1970) who reported free lysine to be present in muscle in high concentrations, thus providing a significant proportion of the daily needs. The importance of the free amino acid pool in skeletal muscle in the economy of total body amino acids is also clearly illustrated in the study of *Pawlack & Pion* (1969). They fed rats for 2 weeks with graded levels of dietary lysine, varying from about one third to twice the amount required for maximum growth. The free lysine concentration in skeletal muscle increased almost 27-fold between the low and high levels of lysine intake, whereas the increase was only 7-fold in the plasma of rats. This again demonstrates the capacity of muscle to act as a store for free lysine. The values summarized by *Munro* (1970b) also show that the proportion of free lysine in muscle is higher than most other dietary essential amino acids. *Hider et al.* (1969) suggested that the greater proportion of amino acids in muscle functions as a body storage pool and that the extracellular pool in this tissue supplies the amino acids for muscle protein synthesis. *Young* (1970) concluded that »protein metabolism in the skeletal muscles is considerably more labile than hitherto appreciated and to a measurable extent this provides the mammal with a significant capacity for adaptation to environmental change«. Provided that these pools are readily available for protein synthesis, the much higher concentration of lysine compared to methionine might imply that experimental animals are less sensitive to a lysine deficient diet than to a methionine deficient diet, at least in experiments of short duration.

D. Balance technique contra total body analysis

Nitrogen retention by animals may be measured by the balance technique or directly by total body analysis. In the nitrogen balance technique the difference between input and output is assumed to be retained in the body. In the total body analysis method the final nitrogen content of the animal is compared with the initial nitrogen content, predicted from analysis of a control group slaughtered at the start of the trial.

These two methods have given results which show close agreement within the range of experimental error (*Becker & Harnisch* 1958a, *Becker & Harnisch* 1958b, *Sanslone & Squibb* 1962, *Nehring et al.* 1964, *Buraczewska et al.* 1969), although discrepancies have been reported which are difficult to explain (*Jakobsen et al.* 1960, *Henry* 1965, *Davidson & Williams* 1968, *Bønsdorff Petersen* 1970, *Just Nielsen* 1970). *Njaa* (1961) pointed out that errors in determining the apparent faecal recovery of ingested nitrogen may result from small losses of both food and faeces and the same would also apply for the urinary nitrogen.

It seems likely that estimation of nitrogen balance is inherently influenced by more numerous sources of error than is the estimation of body nitrogen gain, apart from the fact that the latter estimation is complicated by the use of a zero time control group.

Costa et al. (1968) have suggested that discrepancies between these two methods may be explained if some of the nitrogen fed is eliminated as nitrogen gas. *Lewis & Evans* (1971) have tested this hypothesis by determining nitrogen retention by both methods using rats and chicks in a closed circuit respiration chamber with an argon-oxygen atmosphere. The results showed that neither rats nor chicks liberated gaseous nitrogen from dietary proteins, even when presented with amounts far in excess of requirement. There were no consistent discrepancies between balance and total body analysis methods of estimating nitrogen retention, thus indicating that a high degree of correlation between these two methods can be obtained. Selection of the most suitable method would seem to depend primarily on the precision and reproducibility of measurement and on the time and labour involved. *McLaughlan & Campbell* (1969) concluded that both methods rate the proteins essentially in the same order and that there are usually no real differences in the results obtained.

E. Definition of other biological criteria employed in association with biological value

True digestibility (TD) and BV are considered the main characteristics of a food or feed protein and the net protein utilization (NPU) is considered to be a derived quantity (*Block & Mitchell* 1946–47). In contrast to the apparent digestibility (AD), the true digestibility of a protein source is generally considered to be independent of the protein content of the diet, of the feed intake and of the body weight of the experimental animals (*Allison et al.* 1946, *Mitchell* 1948, *Forbes et al.* 1958, *Njaa* 1959). The influence of dietary protein level on AD, TD, BV and NPU will be discussed in a later section.

The definitions of true digestibility, apparent digestibility and net protein utilization can be expressed in the following equations:

$$\textit{Equation 2: } TD = \frac{\text{N intake} - (\text{faecal N} - \text{metabolic N})}{\text{N intake}} \cdot 100$$

$$\textit{Equation 3: } AD = \frac{(\text{N intake} - \text{faecal N})}{\text{N intake}} \cdot 100$$

$$\textit{Equation 4: } NPU = \frac{TD \cdot BV}{100}$$

If it is desired to combine quality and quantity »utilizable nitrogen« (UN) may be estimated as:

$$\textit{Equation 5: } UN = \frac{NPU \cdot \text{N (in per cent of dry matter)}}{100}$$

In present work UN is calculated together with TD, BV and NPU. Having data for both amino acid composition (AAC) and TD of the individual amino acids, available amino acids (AAA) are obtained by multiplying amino acid composition with the corresponding true digestibility values as follows:

$$\textit{Equation 6: } AAA = \frac{AAC \cdot TD}{100}$$

It will be seen later that AAA is not identical with AAC as considerable amounts of several amino acids escape absorption.

CHAPTER III

Experimental technique for rats

A. Description of the metabolic cage

The metabolic cage employed for rats comprised an upper living area with feeding system and below a device for the collection of urine and faeces. The cage is similar in construction to that described by *Schiller* (1960), although with a modified method for the collection af faeces and urine (*Horszczaruk & Bock* 1963). Since the entire arrangement is modified to a certain extent, a detailed description is given of the cage and its dimensions.

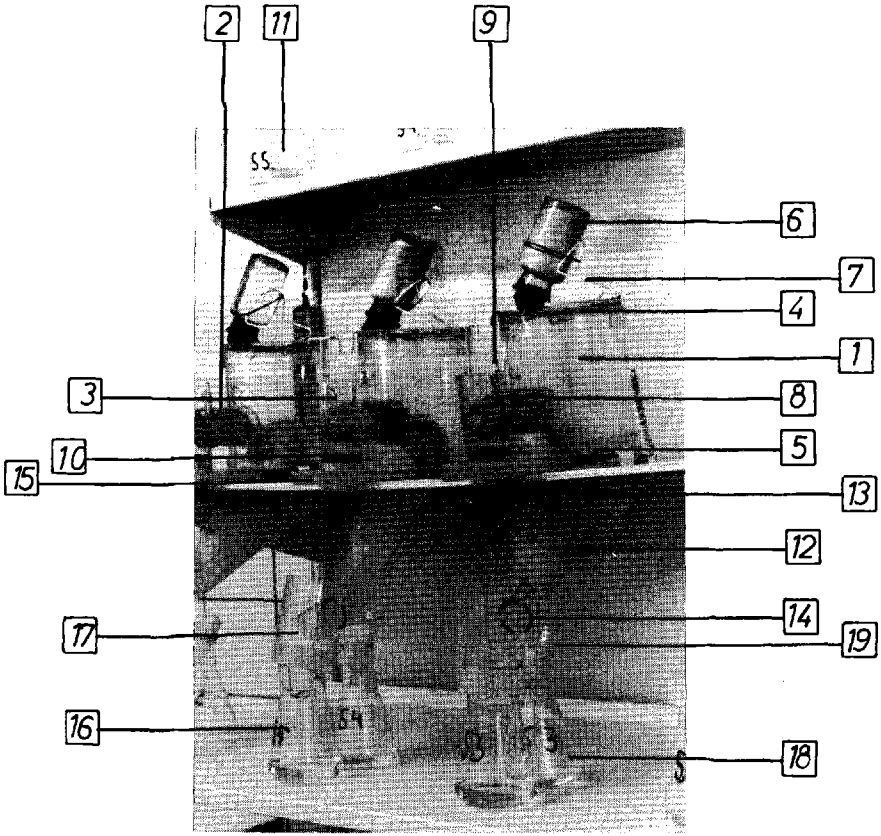

Figure 1. Metabolic cage for rats
Figur 1. Stofskiftebur til rotter

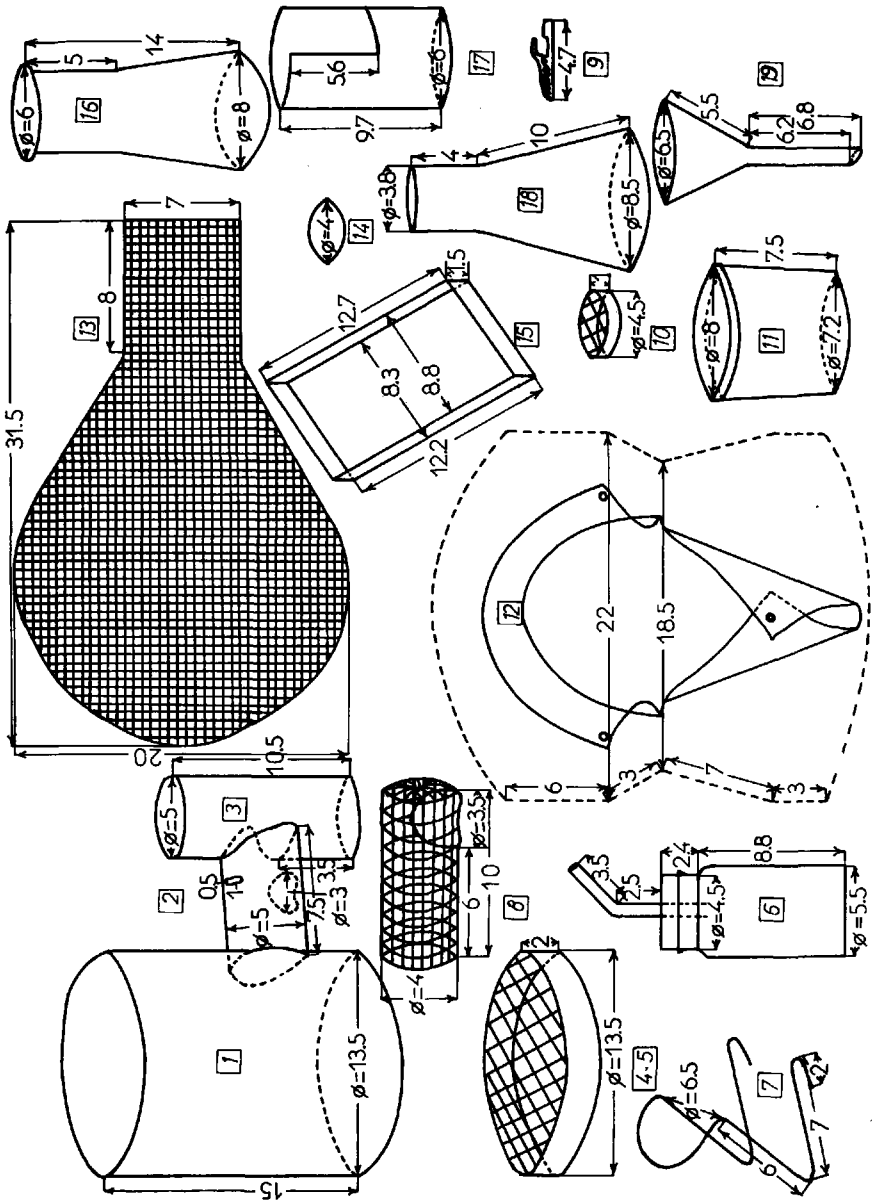

Figure 2. The components of a metabolic cage
Figur 2. De enkelte dele af et stofskiftebur

The functions of many of the components are readily apparent and this description will be limited to certain less obvious parts of the cage.

1. Living area for rats of plexiglass
2. Plexiglass connection to feed box
3. Plexiglass feed box
4–5. Lid and bottom of electroplated brass wire formed as a ring on which a metallic net is soldered
6. Water bottle
7. Metal bracket for water bottle
8. Fine-mesh wire netting covering the connection out to the feed box
9. Clip holding the wire netting in position
10. Diet ring consisting of a brass ring on which electroplated wire netting is soldered
11. Diet box
12. Plastic funnel for collection of urine, placed at an angle of 45°
13. Plastic net for separation of faeces from urine
14. Metallic ring to assemble the plastic net
15. Plastic tray for collecting feed loss
16. Flask for collection of faeces
17. Plexiglass neck-adapter for the faeces flask to prevent loss of faeces
18. Flask for collection of urine
19. Funnel with glass wool to prevent feed, faeces and hair loss in urine

The object of the fine-meshed netting (8) is to reduce the size of the entrance to the feed box and thus force the animals to use their forelegs to get back and forward to the feed. In this way the rats are prevented from carrying feed back and dropping it into faeces and urine. The diet ring (10) prevents the animals from digging in the feed. All components of the metabolic cage are illustrated in Figure 2 and the dimensions are given.

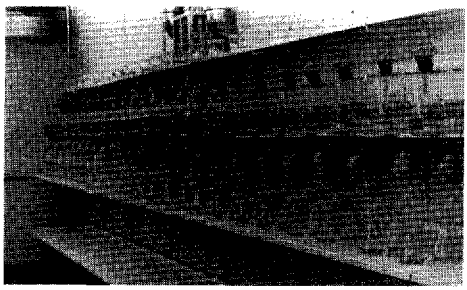

Figure 3. View of the metabolic rat house
Figur 3. Billede fra rottestalden

B. The experimental rats and their diets

Groups of five Wistar male rats weighing approximately 75 g were used in these experiments in which a preliminary period of 4 days and a balance period of 5 days were employed. Each animal received 150 mg N and 10 g dry matter daily throughout the preliminary and balance periods. Feeding took place once a day. The N content was adjusted by using an N-free mixture (see below).

The rats were weighed at the beginning of the experiments and divided into groups of 5 such that the average weights of the groups differed by no more than ± 0.5 g. Weighings were repeated at the end of the preliminary and balance periods; access to feed and water was closed 3 hours before weighing.

The N-free diet had the following composition:

Sucrose	9.00%
Cellulose powder	5.20%
Soya bean oil	5.20%
Potato starch (autoclaved)	80.60%

Autoclaved potato starch was used since crude starch has a negative effect on protein digestibility. Furthermore autoclaving potato starch results in a sandy structure of the material which was found to be of advantage with regard to feeding technique. The diet does not become dusty and does not adhere to spoons, brushes etc., thus aiding quantitative collection. The potato starch was mixed with 1/3 water and autoclaved at 2 atm. for 3 h. and then dried for 3 h.

The mineral mixture comprised the following ingredients and was added to the diet at a concentration of 4%:

Calcium carbonate ($CaCO_3$)	68.6 g
Calcium citrate ($Ca_3C_{12}H_{10}O_{14}$, 4 H_2O)	308.3 g
Calcium hydrogen phosphate ($CaHPO_4$, 2 H_2O)	112.8 g
Dipotassium hydrogen phosphate (K_2HPO_4)	218.8 g
Potassium chloride (KCl)	124.7 g
Sodium chloride (NaCl)	77.1 g
Magnesium sulphate ($MgSO_4$)	38.3 g
Magnesium carbonate ($MgCO_3$)	35.2 g
Ammonium ferric citrate (brown, 20.5–22.5% Fe)	15.3 g
Manganese sulphate ($MnSO_4$, H_2O)	0.201 g
Copper sulphate ($CuSO_4$, 5 H_2O)	0.078 g
Potassium iodine (KJ)	0.041 g
Sodium fluoride (NaF)	0.507 g
Aluminium ammonium sulphate ($AlNH_4(SO_4)_2$, 12 H_2O)	0.090 g

The experimental diet also contained 1% of a vitamin mixture of which 1 kg contained:

1.000 g ≙ 200.000 I.U. vitamin A
0.008 g ≙ 15.000 I.U. vitamin D_3
0.040 g thiamine (vitamin B_1)
0.100 g riboflavin (vitamin B_2)
0.400 g nicotinamide
0.100 g pantothenic acid
0.020 g α-tocopherol (vitamin E)
0.010 g pyridoxine (vitamin B_6)

Autoclaved potato starch was employed in making up to 1 kg. The N-free diet, together with the mineral and vitamin mixture, are standardized mixtures used for many years at the Oskar-Kellner-Institute in Rostock.

The experimental diet was accurately weighed out into plastic boxes with tightly fitting lids for each of the preliminary and balance periods. The daily weighing of feed took place from these boxes in 4 daily allowances in the preliminary period and 5 during the balance period. Any remaining feed was weighed and taken into consideration in the calculation of the experimental data.

C. The collection and analyses of urine and faeces

All parts of the cage which might come into contact with the urine, such as the bottom of the cage, the plastic net and plastic funnel, were greased with vaseline. The plastic net and funnel were also sprayed daily with 20% citric acid to prevent nitrogen loss. For the same reason the glass wool in the urine funnel was sprayed with 5% sulphuric acid.

The urine was collected for all 5 days in the same flask in which 50 ml of 5% sulphuric acid was added. At the end of the experiment all parts which might have come into contact with the urine were washed with approximately 75 ml lukewarm water with a soft brush through a large glass funnel down into the urine flask with funnel and glass wool. The funnel with glass wool was then washed 3 times with water to ensure that all nitrogen had been washed out. The urine + washings were transferred quantitatively to a graduated 300 ml flask and made up with water. The flask was turned several times before 25 ml were removed with a pipette for N determination.

The faeces were collected for all 5 days in 100 ml 5% sulphuric acid. At the end of the experiment 4 × 25 ml concentrated sulphuric acid were added.

After each addition the mixture was stirred thoroughly with a spatula and then left to cool for some hours. When this process is repeated 4 times the

resultant faeces solution is so homogenous that it is possible to take out adequate samples for N determination. Prior to sampling, the mixture was transferred to a 500 ml graduated flask and made up with water. A sample of 100 ml was removed for N determination. All N determinations were carried out according to the Kjeldahl method.

D. Metabolic and endogenous nitrogen as determined in rats

1. General discussion

The importance of the maintenance requirement as a factor in the total protein requirement was emphasized in the extensive work of *Mitchell & Carman* (1924). Their results also led to the general acceptance of the endogenous urinary nitrogen excretion as a measure of the maintenance requirement.

Information on quantity and composition of the various intestinal secretions is rather limited, but there is general agreement that only a small part of these secretions is lost in the stool. The remainder is reabsorbed and represents a mobile protein reserve. This homeostatic mechanism probably serves to even out temporary irregularities in the dietary supply of amino acids and to prevent gross changes in the amino acid pattern of the portal blood.

Nasset (1957) stated that the sources of metabolic protein are the digestive secretions and the mucosal cells which are constantly sloughed.

The subdivision of urinary nitrogen excretion into endogenous and exogenous fractions (*Folin* 1905, *Mitchell* 1955) is accepted by most investigators concerned with protein utilization. But the question of the constancy of the endogenous fraction during periods of protein feeding (*Mitchell* 1948) and its magnitude is still under discussion (*Schoenheimer* 1942, *Frost* 1950, *Bigwood* 1952, *Njaa* 1963).

Njaa (1963) suggested that the measurement of the endogenous N excretion by the original *Mitchell* method (1924a), i.e., the N excreted by animals fed an N-free diet, may introduce considerable and possibly irrelevant variation into the calculation of biological value. *Njaa* showed that the urinary N excreted by animals fed an N-free diet did not remain constant after a period of adaptation, but varied with time and in response to alterations in diet and physiological status of the animal. Similar observations had been reported earlier by *French et al.* (1941). Further doubt concerning the constancy and applicability to BV determinations of the urinary N of animals fed an N-free diet was cast by the results of comparative studies by *Chalupa & Fisher* (1963) and *Henry* (1965). These workers found in general higher net protein utilization (NPU) values for proteins evaluated by the *Thomas-Mitchell* balance sheet method as compared with the *Miller & Bender* (1955) carcass N retention method. Similar results were obtained by *Schiemann et al.* (1963)

with an average difference of 2.7% between the two methods in a series of N balance experiments. As discussed above higher values obtained by the N balance method might be expected since complete urine and faeces collection is extremely difficult to accomplish. This will also apply to total body N analyses, but in this case the values will be too low. This renders complete agreement between the two methods extremely difficult. The N balance method according to *Mitchell* may therefore tend to give excessively high values. However, the accuracy of the method is chiefly dependent on the precision throughout the entire N balance procedure. Even if the NPU values obtained with the N balance method are slightly higher than the »true« NPU values they will nevertheless provide valuable relative figures.

Several factors affect metabolic and endogenous nitrogen, some of which are discussed below.

Results reported by *Bosshardt & Barnes* (1946) indicate that metabolic faecal nitrogen values determined with protein-free or low protein diets are not reliable indices of the metabolic faecal nitrogen under conditions of protein feeding. *Mitchell & Bert* (1954), however, considered the direct determination of the metabolic faecal nitrogen per unit of dry food consumed a valid method for the growing rat. Different proteins in the diet did not appreciably increase (or decrease) the metabolic output of nitrogen. It may reasonably be inferred that the inclusion of protein in the diet did not disturb the ratio of metabolic faecal nitrogen to the dry matter consumed. *Burroughs et al.* (1940) found no evidence to suggest that the endogenous metabolism could be depressed by nitrogen-containing supplements. They concluded that the independence of the endogenous and exogenous types of nitrogen metabolism was thus confirmed.

Causeret et al. (1965) studied the influence of body weight, dry matter intake and dry weight of faeces on the excretion of N. All 3 variables were positively correlated with output of N in faeces, but the closest correlation was found between weight of faeces and N output. All 3 variables were taken into account by means of a regression equation. For certain groups a positive correlation was found between N in urine and body weight or dry matter intake, the former being the more significant and a multiple regression equation gave the most precise estimate. At no time was the precision for endogenous urine N equal to that for metabolic faecal N.

Njaa (1963) indicated that the heavier rats excreted more nitrogen in the faeces than the lighter rats and stated that the faecal nitrogen excretion increased by between 0.04 and 0.08 mg/g rat/day. Although this may be negligible when the body weight differences are small, it may be of importance when larger differences are involved. *Njaa* adds that it is not possible to draw any definite conclusion as to whether the body weight or the growth rate is the more important factor in this connection.

Endegenous nitrogen can be determined by hunger experiments, but here attention has to be paid to the influence of the fattening condition of the experimental animals (*Jakobsen* 1958, *Njaa* 1963). Lean animals have to catabolize tissue proteins and oxidize amino acids to cover their maintenance requirement. Thus nitrogen excretion into the urine will be higher in such animals than in fat animals oxidizing fat.

As previously mentioned, a method widely used is the feeding of experimental animals with an N-free diet sufficient in all other nutrients. Results for metabolic and endogenous nitrogen set in relation to body weight when the rats were fed an N-free diet are summarized below. Metabolic and endogenous nitrogen are expressed in mg, while body weight (x) is expressed in g.

Metabolic N	$= 0.7096 \cdot x^{0.7555}$	(*Columbus* 1954)
»	$= 0.05561x + 16.8$	(*Nehring & Haesler* 1954)
»	$= 0.141x + 7.59$	(*Bock* 1958)
»	$= 0.177x - 5.621$	(*Bock et al.* 1964)
»	$= 0.071x + 3.065$	(*Causeret et al.* 1965)
»	$= 0.081x + 3.01$	(*Lehmann et al.* 1968)
Endogenous N	$= 1.438 \cdot x^{0.601}$	(*Columbus* 1954)
»	$= 0.2072x - 1.7$	(*Nehring & Haesler* 1954)
»	$= 0.169x + 3.74$	(*Bock* 1958)
»	$= 0.442x - 4.634$	(*Bock et al.* 1964)
»	$= 0.2077x + 6.728$	(*Causeret et al.* 1965)
»	$= 0.147x + 19.43$	(*Lehmann et al.* 1968)

As can be seen from the above equations, there are considerable discrepancies from one laboratory to another regarding the influence of body weight on metabolic and endogenous nitrogen.

Causeret et al. (1965) did not find dry matter consumption to influence metabolic N, whereas such an effect was reported by *Bock et al.* (1964). *Mangold & Behm* (1955) and *Mitchell & Carman* (1926) also found dry matter consumption to influence metabolic N.

Egg protein is usually considered to be completely digested and utilized (NPU = 100). This fact is taken into account in estimating metabolic and endogenous nitrogen, since nitrogen excreted in faeces and urine when a low egg protein diet is fed must originate from the body. The use of egg protein for this purpose has been comprehensively and well reviewed by *Njaa* (1963).

In order to illustrate the magnitude of metabolic nitrogen estimated on low egg protein diets, results from the study of *Njaa* (1963) and others are listed in Table 1. The figures are quoted from a table of Njaa.

Table 1. Faecal nitrogen in mg/g feed consumed on low egg protein diets
Tabel 1. Gødningskvælstof i mg/g konsumeret ægprotein

Feed intake (g/day)	Body weight (g)	mg faecal N/ g feed intake	References
8.0	114.7	1.50	*Mitchell & Carman* 1926
7.3	76.4	2.38	*Bartlett et al.* 1938
8.6	114.5	2.43	*Macrae et al.* 1943
8.7	96.5	2.08	*Henry & Kon* 1946
9.8	100.0	2.16	»
8.1	81.8	2.12	»
10.0	100.8	1.82	*Njaa* 1963
9.0	94.7	1.84	»
10.0	85.5	2.02	»

These results show a certain degree of variation in metabolic nitrogen expressed in mg faecal N/g feed intake. Within the same laboratory, however, the differences are relatively small. In most cases metabolic nitrogen is in the range of approximately 2 mg N per g feed eaten. Furthermore there appears to be no relationship between body weight and metabolic N excretion when expressed in this way.

In order to compare values of metabolic N obtained on low egg protein diets, values estimated on N-free diets (*Njaa* 1963) are shown in Table 2.

Table 2. Faecal nitrogen in mg/g feed consumed on N-free diets
Tabel 2. Gødningskvælstof i mg/g konsumeret N-frit foder

Feed intake (g/day)	Body weight (g)	mg faecal N/ g feed intake	References
6.62	129.5	1.90	*Mitchell & Carman* 1926
8.5	40–100	2.01	*Columbus* 1954
12.1	100–150	2.34	»
13.6	150–180	2.35	»
15.3	180–250	2.66	»
6.78	65	2.14	*Behm* 1955
4.69	87.5	1.94	»
7.69	102.3	2.17	»
2.95	110.6	2.62	»
12.75	169.6	2.50	»
9.38	184.5	2.29	»
14.66	194.4	2.15	»
5.90	202.5	2.90	»

The results of *Columbus* (1954) appear to indicate that mg faecal N/g feed eaten increases to a certain extent with increasing body weight and feed consumption. *Behm* (1955), however, found no such relation.

A comparison of the results in Table 1 and 2 shows that data for metabolic N obtained on N-free diets are slightly higher than data from low egg protein diets. This would suggest that egg protein is completely digested and thus the use of egg protein in the diet for estimating metabolic nitrogen can be regarded as a reliable technique. Furthermore there can be little doubt that a more favourable physiological state of the experimental animal is achieved when a certain level of protein is fed than when an N-free diet is employed.

The evidence of complete digestibility of egg protein is somewhat conflicting; *Mitchell & Carman* (1926), who introduced the low egg protein diet as a standard diet instead of the N-free diet, concluded that there was practically no difference between faecal nitrogen excretion per gram of ingested feed in the two types of diets. However, *Bosshardt & Barnes* (1946) working with mice and *Mitchell & Bert* (1954) and *Nehring & Haesler* (1954) with rats have shown that whole egg protein is not completely digestible.

The disagreement concerning the ability of rats to utilize egg protein might well be due to the quality of egg protein employed. In the present experiments (see later) it was found important to extract the fat before use, since excretion of nitrogen to the faeces was 4 to 5% higher when unextracted egg protein was used compared to ether extracted protein. In addition the eggs should be freeze-dried in order to prevent the exposure of the protein to heat.

With regard to the endogenous urinary excretion, *Njaa* (1963) found the level of excretion to vary both with the body weight and the growth rate of the rats. Similar results were obtained by *Mitchell & Carman* (1926). However, *Zimmermann* (1952) failed to find a significant relationship between endogenous urinary nitrogen excretion and body weight.

2. Present investigations

a. Procedure for determination of metabolic and endogenous nitrogen

To illustrate the above problems a diet containing 4% freeze-dried, ether extracted egg protein was fed to 40 rats. Dry matter consumption averaged 9.12 g/animal/day. Nitrogen excreted with the faeces made up 2.04 ± 0.04 mg/g dry matter consumed, while the nitrogen excreted with the urine amounted to 15.2 ± 0.21 mg per rat/day. Provided that this egg protein was completely utilized in the body, these values should be identical with metabolic and endogenous nitrogen respectively. In order to test this hypothesis of 100% utilization a second experiment was carried out with 9.36% egg protein in dry matter fed to 10 rats. TD and BV were calculated by using the factors

for metabolic and endogenous nitrogen in the experiment with 4% egg protein in the diet. The results from this experiment are shown below.

$$\begin{aligned} TD &= 100.6\% \pm 1.6 \\ BV &= 98.6\% \pm 1.9 \\ NPU &= 99.5\% \pm 2.1 \end{aligned}$$

TD and BV can be seen to approximate 100%, thus indicating that carefully treated egg protein may be completely utilized by young fast growing rats. This is in accordance with results obtained with rats by *Nehring & Haesler* (1954). They found a close correlation between metabolic and endogenous nitrogen calculated on N-free and 4.9% egg protein diets respectively. By increasing the concentration of egg protein, however, BV decreased.

To provide additional information concerning the digestibility of egg protein, five faeces samples from the diets with 4.00 and 9.36% egg protein were analysed for amino acids. Assuming the amino acid composition of metabolic protein and egg protein to be different, the amino acid analyses would disclose possible differences in the amino acid digestibilities and consequently protein digestibility.

In the following table the average amino acid analyses of five faecal proteins obtained when the rats were fed diets containing 4.00 and 9.36% egg protein respectively are shown. For comparison the analysis for egg protein is listed.

As shown in Table 3, the amino acid composition in rat faeces was found to be very much the same despite the level of egg protein in the diet. This would appear to indicate that all amino acids in egg protein are completely absorbed. If the amino acids were not completely digested the amino acid composition of rat faeces would be expected to show a closer resemblance to egg protein at the 9.36% level than at the 4.00% level. However, this was not found to be the case. Furthermore the nitrogen present in the form of amino acids is higher in egg than in faeces, g/16 g N being higher for almost all amino acids in egg protein. This would indicate that the body secretes non-amino N constituents into the digestive tract or that non-amino N constituents are less absorbable than amino N. This is also indicated by the higher content of ammonia in rat faeces compared to egg, although this difference might equally well be due to deaminising processes from the microbial flora. The significance of the bacterial flora will be discussed in a later section.

The high content of serine in faeces should be noted since this will affect the TD values for this amino acid in a positive direction as will be seen later. It is unlikely that the high serine content is due to a poor digestibility of this amino acid in egg protein since the content in faeces is the same at both levels of egg protein in the diet. On the other hand *Meier et al.* (1970b) found a low TD value for serine in egg protein. Furthermore present

Table 3. Amino acid composition of faeces protein at two different dietary levels of egg protein

Tabel 3. Aminosyresammensætningen i gødningsprotein ved to forskellige niveauer af ægprotein i foderet

Egg protein in diet (%)	4.00 (g/16 g N)	9.36 (g/16 g N)	Egg protein (g/16 g N)
Lysine	5.88	5.77	6.65
Methionine	2.00	1.93	3.01
Cystine	1.92	1.76	2.33
Aspartic acid	8.86	9.07	10.36
Threonine	4.61	4.79	5.14
Serine	7.71	7.72	7.72
Glutamic acid	10.31	10.88	14.68
Proline	3.21	3.19	4.21
Glycine	4.11	4.15	3.59
Alanine	5.03	4.78	6.11
Valine	4.55	4.49	7.54
Isoleucine	3.75	3.77	5.76
Leucine	5.45	5.68	8.90
Tyrosine	3.82	3.49	3.63
Phenylalanine	3.92	4.32	6.69
Histidine	2.21	2.21	2.54
Arginine	4.18	4.47	6.15
Tryptophan	1.13	1.16	1.49
Ammonia	1.68	1.71	0.96

observation is not in agreement with the work of *Slump* (1969) who found considerably more serine in faeces when egg protein was fed compared to protein-free diets. The same tendency was found for histidine, while no significant differences were found between a nitrogen-free diet and a 10% egg protein diet with regard to other amino acids (*Slump* 1969). Slump presented his results as g amino acids per 100 g dry faeces and it is of interest that the amino acid composition of metabolic protein is relatively constant. *Slump* (1969) analysed faeces from 8 groups of rats, 4 groups on N-free diets and 4 groups on 10% egg protein diets.

On the basis of the results obtained in the present investigations and those referred to in the literature it was considered advisable to employ the data for metabolic and endogenous nitrogen obtained on a diet low in egg protein (4%) in the calculation of TD, BV and NPU. In order to determine the validity of these constants under the experimental conditions employed the influence of body weight and feed residues were examined.

b. The influence of body weight on metabolic and endogenous nitrogen excretion

As previously discussed, body weight may affect both metabolic and endogenous nitrogen excretion, i.e., heavier animals will excrete more than lighter animals despite similar levels of dry matter intake. Consequently heavier animals will give lower values for both TD and BV provided that constant factors are used for metabolic and endogenous nitrogen irrespective of body weight.

In the present study animals were required with a body weight of approximately 75 g at the beginning of the experiments, although variation in body weight of 5–6 g within groups was often unavoidable. In order to determine whether weight differences of this magnitude affect TD and BV, a t-test was made between 40 random pairs of rats from 40 different diets (*Eggum* 1968a). The weight difference between the two rats in each group was at least 5 g. In the calculation of TD and BV the same constants for metabolic and endogenous nitrogen were employed irrespective of body weight.

However, these calculations showed no statistical differences ($P > 0.05$) in TD or BV values between the two groups of rats. A t-value of 0.121 was obtained for TD values. When the TD values for the smaller animals were subtracted from those of the heavier animals the sum of differences (Σd) was only 1.44.

In the case of BV a t-value of 0.312 was obtained, although the sum of the differences (Σd) was –8.25. This would indicate that BV tends to decrease with increasing body weights in agreement with the results obtained by other workers. However, as these differences were far from statistically significant, no change was made in the correction factors for either endogenous or metabolic nitrogen with regard to the small differences in body weight of the experimental rats employed in the present work.

From this discussion it would appear that differences in body weight within the weight-range encountered in the present experiments do not influence the TD and BV values significantly.

c. The influence of feed residues on true digestibility and biological value

In biological experiments problems due to feed residues are frequently encountered and necessitate correction.

In order to test the level of metabolic N, TD values from a further random 40 pairs of rats with and without feed residues were compared, i.e., TD from a rat in one group with feed residue was compared with TD from a rat in the same group without feed residue. A t-test on these 40 pairs showed $P > 0.05$ and t was 0.229. Thus there were no significant differences between TD values obtained from animals with and without feed residues. It should be noted that metabolic nitrogen is calculated as directly dependent on dry

matter consumed, i.e., 2.04 mg N/g dry matter. From the above calculations the sum of the differences (Σd) obtained by subtraction of TD values for rats with feed residues from rats without was 5.15. This suggests that animals with feed residues tended to give lower TD values than animals without. Consequently the factor for metabolic nitrogen would appear to be slightly higher than the »true« value, although the calculations do not indicate any significant influence on TD values.

To test the validity of the correction factor for endogenous nitrogen, similar calculations were carried out for BV using 40 pairs of rats with and without feed residues. No significant differences in BV values were found between rats with feed residues compared to rats without ($P > 0.05$, $t = 0.449$). This would suggest that the BV values are independent of feed residues. However, the sum of the differences (Σd) in BV values between animals with and without feed residues was 12.68, indicating that the correction factor for endogenous nitrogen is too high as rats with feed residues tend to give lower BV values than rats without. Since the statistical calculations show no significant difference a constant factor of 76 mg N/rat/5 days was employed for endogenous nitrogen irrespective of feed residues. It should, however, be emphasized that in groups with feed residues the standard deviation is normally higher than in groups without.

These comparisons of results from rats with and without feed residues indicate that the correction factors obtained for metabolic and endogenous N appear to be reliable for the calculation of TD and BV.

CHAPTER IV

Experimental technique for baby pigs

A. Description of the metabolic cage

A metabolic cage, 200 cm. long, 80 cm. wide and 50 cm. high, was employed in the following experiments with baby pigs. As this cage is identical with that described by *Ludvigsen & Thorbek* (1959, 1960) only few comments will be given. The cage is depicted in Figure 4.

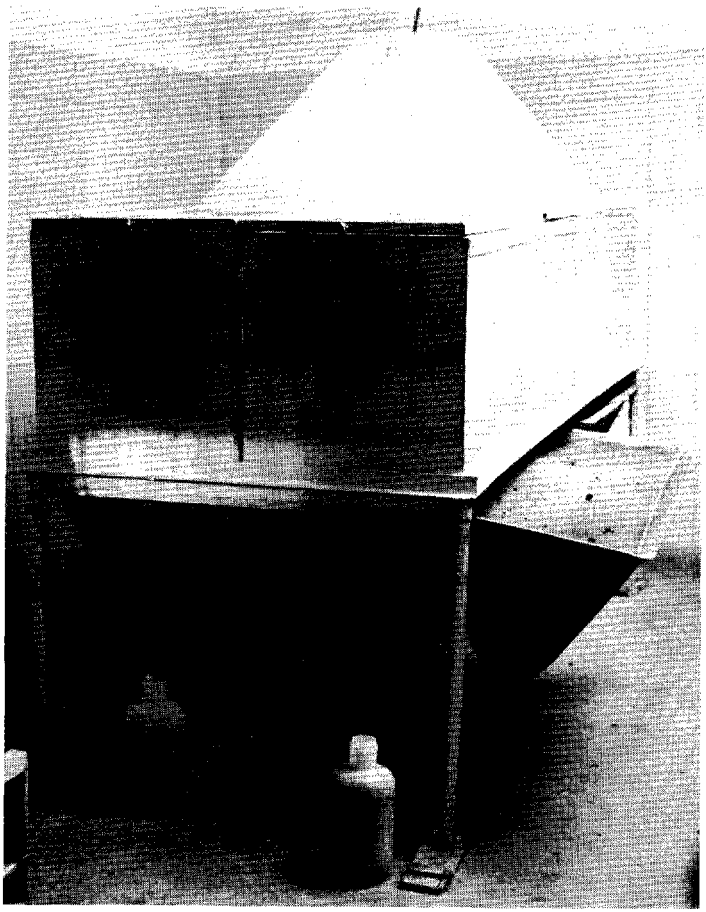

Figure 4. Metabolic cage for baby pigs
Figur 4. Stofskiftebur til pattegrise

The cage is divided into four sections with room for four separately housed pigs. The system employed to separate faeces and urine is approximately the same as that described for rats; the bottom of the cage consists of a metallic net 1 × 1 cm. beneath which is located a fine wire mesh for the collection of faeces while the urine is collected by means of an acryl funnel and carried off to a urine flask.

B. The experimental pigs and their diets

In these experiments male pigs of Danish Landrace were employed if nothing else is mentioned. The pigs were weaned at an age of 10 days (about 3.5 kg) and fed a milk substitute (Rød laktal) for 6 days. Weaning was generally accomplished without difficulty and the animals started to gain weight after 3–4 days. The pigs were fed 6 times daily, starting at 6.00 a.m. Feeding took place outside the cage in order to prevent feed loss into urine and faeces. The feeding arrangement is shown in Figure 5.

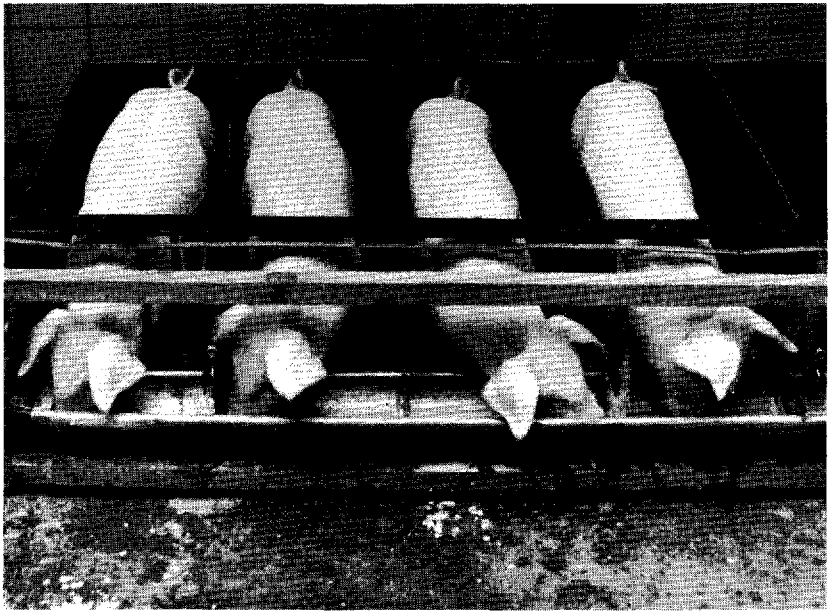

Figure 5. Feeding arrangement outside the cage
Figur 5. Fodringssituation uden for buret

After the feed ration had been consumed the troughs and the noses of the pigs were rinsed by spraying with water which the pigs were allowed to drink. In this way an almost complete feed consumption was possible.

Feed and water were administered according to the method described by *Ludvigsen & Thorbek* (1959, 1960), i.e., gradual daily increases. The feed plan is given in Table 4. This plan was strictly followed in all the following experiments, although the age of the pigs can differ by one or two days from one experiment to another.

The feed was thoroughly mixed in lukewarm water before feeding. Due to the daily increase in feed quantity, the collection of faeces and urine was delayed by one day. Thus in a balance period of 4 days the feed consumed on the 6th, 7th, 8th and 9th day corresponded to the faeces and urine excreted on the 7th, 8th, 9th and 10th day. This procedure was employed in all experiments.

After 6 days of habituation to »Rød laktal« the pigs were put on 3 × 3 days N balance periods to check the individual variation. While waiting for the chemical analyses the pigs were fed »Rød laktal« for further 5 days. Provided that the variation was within a normal range the pigs were then fed the experimental diet for a preliminary 3-day period and subsequently a 4-day balance period. The experimental period was followed by a further 5 days on »Rød laktal« without faeces and urine collection. This procedure was repeated 3 times, giving 12 observations per diet since 4 pigs were employed per group. The balances were thus distributed with the first period at approximately 30, the second at 42 and the third at 54 days of age.

The composition of the milk substitute (Rød laktal) was as follows:
Mælkeerstatningen (Rød laktal) havde følgende sammensætning:

Oat meal	53.3%
Skim milk powder	33.3%
Dried blood plasma from pigs	4.4%
Lard	4.4%
Soya oil	2.2%
Mineral mixture[1])	2.2%
Vitamins[2])	0.2%
	100.0%

[1]) Mineral mixture:

Dicalcium phosphate ($CaHPO_4$, 2 H_2O)	50.00%
Calcium carbonate ($CaCO_3$)	39.35%
Ferric lactate ($Fe(C_3H_5O_3)_3$)	6.75%
Manganese sulphate ($MnSO_4$, H_2O)	3.00%
Zinc sulphate ($ZnSO_4$)	0.40%
Copper sulphate ($CuSO_4$, 5 H_2O)	0.32%
Cobalt sulphate ($CoSO_4$)	0.09%
Magnesium sulphate ($MgSO_4$)	0.09%

Table 4. The standard feed plan used in all experiments with baby pigs
Tabel 4. Standardfoderplan for alle forsøgene med pattegrise

Age of the pigs (days)	Feed per day (g)	Water per feeding (ml)
10	60	30
11	72	36
12	84	42
13	96	48
14	108	54
15	120	60
16	135	78
17	150	88
18	165	96
19	180	105
20	195	114
21	210	123
22	225	131
23	240	140
24	255	149
25	270	158
26	285	166
27	300	175
28	318	186
29	336	196
30	354	207
31	377	217
32	390	228
33	408	238
34	426	249
35	444	259
36	462	270
37	480	280
38	498	291
39	516	301
40	537	313
41	558	326
42	579	338
43	600	350
44	621	362
45	642	375
46	663	387
47	684	399
48	705	411
49	726	433
50	747	455
51	768	477
52	789	499
53	810	521
54	831	543
55	852	565

[2]) To each gram of the milk substitute was added:

Vitamin A	10 I.U.
Vitamin D_3	3.3 I.U.
Riboflavin	4 microgram
Nicotin amide	12 »
d-Pantothenic acid	70 I.U.

Since the pigs appeared to do well on »Rød laktal«, an attempt was made to adjust the experimental diets to the same content of fat, oil, minerals and vitamins as contained in this milk substitute. Crude fibre was adjusted to 4% by the use of cellulose powder while the N concentration was adjusted by means of a mixture of 80% maize starch and 20% sucrose.

Since all diets were prepared according to the above procedure, the detailed composition for each diet will not be given.

In experiments with concentrated feeds the N concentration was adjusted to 3.0% of dry matter. Feeds with a lower nitrogen content could not be made up to this N content when only one nitrogen-carrying substance was employed. Thus the cereals were adjusted to a lower N content – 1.50% of dry matter.

As will be discussed in a later chapter, the N content of the diet affects the BV value and thus a comparison of BV values obtained at different N levels is incorrect. The main reason for the different N levels used in the present experiments is that appetite problems were expected on diets low in nitrogen. The experiments were therefore started with concentrated feeds which were adjusted to 3.0% nitrogen. In the case of cereals, however, it was shown to be possible to induce the piglets to accept diets low in nitrogen for a week at the time. However, feed residues could not be completely avoided and probably contribute to the relatively large standard deviations found for certain of the results.

C. The collection and analysis of urine and faeces

As mentioned above, faeces were collected on a fine wire mesh placed beneath the bottom of the cage while the urine was collected by a funnel and run off to a flask. The faeces were picked up from the wire mesh twice daily and stored in a refrigerator at 4°C for the entire period. All faeces from each pig during one period were collected in one box and an N determination made on a homogenate of this material. On the basis of the weight of faeces in each box, the amount of faeces N excreted per pig in one period could thus be estimated.

To prevent urine from sticking to the acryl funnel frequent rinsing with water was carried out. At the end of each period all equipment which might

have been in contact with the urine was washed and the washings collected with the urine. The pH in the urine flask was maintained slightly acid by the use of a solution of 5% sulphuric acid. The urine flask was emptied twice daily and the urine stored in a larger flask in the refrigerator.

At the end of the period the volume of urine, the N content, and hence the total amount of nitrogen excreted with the urine were determined.

Having obtained values for the amount of nitrogen consumed, metabolic N, endogenous N, and the amount excreted with faeces and urine respectively, digestibility as well as biological value and net protein utilization could be estimated.

D. Metabolic and endogenous nitrogen as determined in pigs

1. General discussion

In the experiments on protein utilization reported in this chapter the conclusions are based upon the nitrogen metabolism of growing pigs under conditions of dietary control, in accordance with the method developed by *Mitchell* (1924a) from the early studies of *Thomas* (1909) and used in previous experiments with pigs by *Mitchell & Kick* (1927) and *Mitchell & Hamilton* (1931).

Although the method has been used rather extensively, its basic tenets have not gone unchallenged through the years. The objection that the faecal nitrogen output on a nitrogen-free diet is not a reliable measure of the metabolic faecal nitrogen has been shown to be groundless for animals other than the mouse by *Mitchell & Bert* (1954), and evidence to the same effect has been offered by *Armstrong & Mitchell* (1955) for the pig.

In order to demonstrate the size of the metabolic nitrogen fraction values determined at different laboratories by two different experimental techniques – direct estimation and extrapolation – are shown in the following table. Most of the values are quoted from tables given by *Dammers* (1964) and *Wiesemüller & Poppe* (1969a).

In the direct method the animals were fed an N-free diet. Consequently N in faeces must originate from the organism and be identical with the metabolic nitrogen. In the extrapolation method, however, the animals were fed increasing amounts of protein which were not completely digested. Thus the excretion of nitrogen with the faeces will increase with increasing nitrogen in the diet. By plotting the nitrogen excretion against the nitrogen intake metabolic nitrogen is obtained by extrapolation to zero nitrogen intake.

These two methods of determining metabolic nitrogen will not be discussed here; the results of the previously described experiments with rats led to the use of 4% freeze-dried, ether-extracted egg protein in diets for estimating

Table 5. Metabolic nitrogen of pigs expressed in g per 100 g dry matter consumed
Tabel 5. Stofskiftekvælstof hos grise udtrykt i g pr. 100 g fortæret tørstof

	g metabolic N per 100 g dry matter consumed	Technique
Armstrong & Mitchell (1955)	0.091	Extrapolation
ibid.	0.114	Direct
Bell et al. (1950)	0.180	Extrapolation
Carlson & Bayley (1970)	0.111	Direct
Columbus (1950)	0.09	Direct
ibid.	0.189	Direct
Dammers (1964)	0.177	Direct
Hennig (1957)	0.119	Direct
Hennig (1959)	0.151	Direct
Homb (1962)	0.198	Extrapolation
Lintzel & Mangold (1935)	0.095	Direct
Mangold & Behm (1955)	0.105	Direct
ibid.	0.130	Direct
ibid.	0.199	Direct
Nehring & Schramm (1940)	0.155	Direct
Nehring (1953)	0.176	Direct
Schiftan (1933)	0.096	Direct
Wiesemüller & Poppe (1969a)	0.157	Extrapolation
Zausch (1954)	0.189	Direct
Zelter & Charlet-Lery (1961)	0.194	Direct

metabolic and endogenous nitrogen. However, there do not appear to be pronounced differences in the data in Table 5 due to the different methods. Many of the data differ considerably, although the majority are within the same range. The results of *Mangold & Behm* (1955) were obtained at different concentrations of crude fibre and the authors concluded the increase in metabolic N to be due to an increase of crude fibre in the diet. As going to be discussed later this is not in agreement with present results obtained with rats.

The difficulty in measuring »valid« endogenous nitrogen output in the urine of pigs has been exaggerated by *Bell et al.* (1950) and *Bell & Loosli* (1951) in the face of the earlier experiences of *McCollum & Steenbock* (1912), *Lund* (1935) and of *Smuts* (1935). *Wiesemüller & Poppe* (1969a) found an average of 0.239 g N/kg $W^{0.67}$ for several feeds when the extrapolation technique was employed. The authors were, however, somewhat sceptical about this procedure. Calculations of *Wiesemüller & Poppe* (1969a) showed that in experiments by *Columbus* (1950) endogenous urine N levels of 0.160 g N/kg $W^{0.67}$ were obtained, while *Armstrong & Mitchell* (1955) found 0.106 g N/kg $W^{0.734}$. It appears that the values for endogenous nitrogen differ considerably from

one institute to another. This would appear to indicate difficulties in obtaining a reliable estimate of this factor.

2. Present investigations

As previously stated, metabolic and endogenous nitrogen were measured in the present investigation on a diet containing 4% freeze-dried, ether-extracted egg protein. In using this method it is assumed that baby pigs (as well as rats) utilize egg protein completely. Four piglets were used in this work and all were measured in three 3-day periods as described earlier. In the following calculations the quantities of nitrogen excreted with faeces and urine respectively are related to the dry matter consumed. It should be stressed that feed consumption is related to the age of the pigs and consequently to body weight. In Table 6 the consumption of dry matter and the excretion of nitrogen in both faeces and urine are shown for pigs fed a diet with 4% freeze-dried, ether-extracted egg protein.

Table 6. The relationship between dry matter consumption (and body weight) and nitrogen excreted in faeces and urine

Tabel 6. Forholdet mellem fortæret tørstof (og dyrenes vægt) og kvælstof udskilt med gødning og urin

Pig no.	Body weight (kg)	Dry matter consumption (g/day)	Nitrogen in faeces (mg/day)	Nitrogen in urine (mg/day)
1	4.86	257	310	371
2	5.51	257	224	349
3	6.54	257	219	427
4	6.63	257	292	440
1	7.45	396	310	500
2	8.03	396	261	520
3	9.19	396	366	683
4	9.35	396	305	625
1	10.76	616	463	843
2	11.51	616	439	717
3	12.43	616	448	869
4	13.21	616	392	781

It is clear from Table 6 that a linear relationship exists between dry matter consumption (and body weight) and the nitrogen excreted in faeces and urine respectively. The regression equation for N in faeces was calculated as:

Equation 7:

$$\text{mg N in faeces} = 127.26 + 0.49 \cdot \text{g dry matter consumed}$$

$$s = 39.68, \quad s_b = 0.08, \quad r = 0.90$$

The regression coefficient differs significantly from zero ($P < 0.001$). Thus metabolic nitrogen increases when dry matter consumption increases. From the regression equation metabolic nitrogen excreted at 100 g dry matter consumed was estimated as 0.176 g. This factor is close to several of the values referred to in Table 5. However, it appears from the regression equation that there is no direct relationship between metabolic nitrogen and dry matter consumed due to the relatively large constant of 127.26.

The relationship between dry matter consumption and the nitrogen in urine is shown in the following regression equation:

Equation 8:

$$\text{mg N in urine} = 120.57 + 1.12 \cdot \text{g dry matter consumed}$$
$$s = 66.53, \quad s_b = 0.13, \quad r = 0.94$$

The correlation obtained can be regarded as a »false« correlation, since an almost linear relationship exists between dry matter consumed and the weight of the pigs. The regression coefficient, however, is significantly different from zero ($P < 0.001$). The high correlation coefficient (0.94) would appear to indicate that endogenous nitrogen increases linearly with dry matter consumption in the range studied.

The relationship between body weight and nitrogen excreted into the urine is given by the following regression equation:

Equation 9:

$$\text{mg N in urine} = 33.99 + 63.69 \cdot \text{body weight (kg)}$$
$$s = 60.20, \quad s_b = 6.61, \quad r = 0.95$$

The regression coefficient obtained by this estimate was also found to be significant ($P < 0.001$). Due to the relationship between dry matter consumption and body weight, the excretion of endogenous nitrogen would appear to show a linear response to both body weight and dry matter consumption under the range of body weights studied (4–14 kg). This is not in agreement with the values reported by *Wiesemüller & Poppe* (1969a) who found a curvilinear relationship between endogenous N and body weight.

The relationship between dry matter consumption and nitrogen excreted with faeces and urine respectively is illustrated in Figure 6.

In the following experiments metabolic nitrogen and endogenous nitrogen are estimated from the equations shown in Figure 6.

The amounts of amino acids of metabolic origin are calculated in the same manner as metabolic nitrogen, i.e., they are estimated in relation to dry matter consumption (see later). The amino acid composition of metabolic protein from the present investigations with pigs is given in Table 7 together with

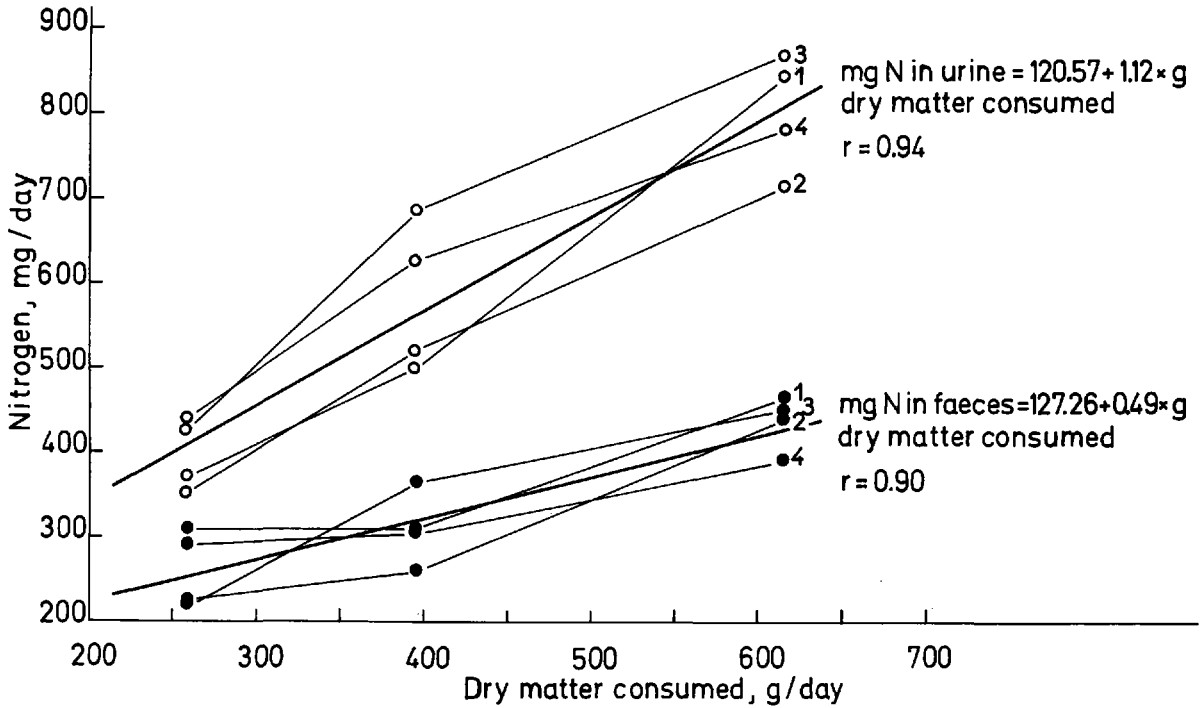

Figure 6. Nitrogen in faeces and urine in relation to dry matter consumption
Figur 6. Kvælstof i gødning og urin i relation til fortæret mængde tørstof

Table 7. Amino acid composition of metabolic protein determined at different laboratories

Tabel 7. Aminosyresammensætning i stofskifteprotein bestemt på forskellige laboratorier

Source	*Dammers* (1964)	*Carlson & Bayley* (1970)	*Eggum* (1971)	*Eggum* (1971)
Animal species	Pigs (g/16 g N)	Pigs (g/16 g N)	Pigs (g/16 g N)	Rats (g/16 g N)
Lysine	4.90	4.30	5.65	5.88
Methionine	2.10	–	2.03	2.00
Cystine	1.50	–	1.61	1.92
Aspartic acid	9.00	8.30	8.34	8.86
Threonine	4.80	5.10	4.38	4.61
Serine	3.80	4.70	7.01	7.71
Glutamic acid	9.40	8.00	10.66	10.31
Glycine	4.20	4.30	4.48	4.11
Alanine	5.30	5.00	5.64	5.03
Valine	5.00	4.90	4.82	4.55
Isoleucine	4.00	4.20	3.97	3.75
Leucine	5.60	6.40	6.07	5.45
Tyrosine	1.80	3.00	3.44	3.82
Phenylalanine	3.60	3.80	5.09	3.92
Histidine	1.40	–	2.09	2.21
Arginine	3.20	–	4.19	4.18

results of other workers. For comparison the results of analyses with rats are also included.

The experiments of *Dammers* (1964) were carried out with adult pigs on an N-free diet, while the experiments of *Carlson & Bayley* (1970) employed baby pigs fed an N-free diet.

It appears from Table 7 that the amino acid composition of metabolic protein assayed at different laboratories shows reasonable agreement. However, diverging values are found in the case of certain amino acids, particularly serine. Estimates of this amino acid in the present investigation are much higher than those of other workers and are certainly incorrect as will be seen later. The high serine values found might be due to the fact that this amino acid is not completely absorbed from egg protein. This is in agreement with previously discussed work by *Slump* (1969) who compared the amino acid composition in the faeces of rats fed an N-free diet and a diet with 10% egg protein. Expressed in gram amino acids per 100 g dry faeces, *Slump* (1969) found that the serine excreted with the faeces was almost twice as high when the rats were fed 10% egg protein in the diet than when they were given an N-free diet. In the case of the other amino acids, this comparison showed a tendency for histidine and arginine contents to be higher when the 10% egg protein diet was fed than when the N-free diet was given; other amino

acids appeared to be independent of the presence and level of egg protein in the diet. This agrees well with the results shown in Table 7; the values obtained by the present author for histidine and arginine in both pigs and rats are higher than the corresponding values obtained by *Dammers* (1964) on an N-free diet. Better agreement is found with the other amino acids.

It is concluded that the present values for serine in endogenous protein are too high and will cause an overestimation of its TD. Overestimation of TD values for histidine and arginine may also result. The factor for the other amino acids should give correct TD estimates irrespective of whether they are obtained on an N-free diet or a diet with egg protein.

As the significance of the bacterial flora is discussed in a later section, this aspect is not taken up at this stage. It should be considered, however, that part of the protein estimated as metabolic protein might well be of bacterial origin. However, comparison of the amino acid composition of faeces protein with the amino acid composition of bacterial protein given by *Weller* (1957) does not indicate a significant contribution of bacterial protein to the faeces protein.

E. The influence of age on protein utilization in pigs

1. General discussion

The influence of age on protein utilization has often been discussed but little experimental work has been done in this field. However, *Bell & Loosli* (1951) demonstrated a marked decrease in calculated BV in growing pigs when a constant feed mixture was fed to animals of increasing size. This is probably due to the fact that the pigs received an increasing level of protein relative to their decreasing requirement as they grew larger. On the basis of balance experiments with 3–7 weeks old pigs, *Lloyd et al.* (1957) concluded that digestibility coefficients of nitrogen were lower during the first weeks of life. The lower digestibility was ascribed to a low level of activity of the proteolytic enzymes. However, this conclusion differs from that of *Ludvigsen & Thorbek* (1959, 1960) who found the digestibility of nitrogen to be independent of the age of the piglets. Recent work by *Homb* (1972) shows that growing barrows from 30 to about 90 kg show an approximately constant efficiency of protein utilization whereas heavier pigs show very great variability in this respect.

Since little precise information is available for the age interval concerned in these investigations, experiments were carried out with pigs 20–55 days old in order to determine the validity of direct comparisons of results obtained with animals within this age range.

To elucidate this aspect three N balance experiments were carried out

with artificially reared baby pigs. Apparent nitrogen digestibility (AD) and retention coefficients (RC) of nitrogen were employed as criteria:

Equation 3:

$$AD = \frac{(\text{N intake} - \text{faecal N})}{\text{N intake}} \cdot 100$$

Equation 10:

$$RC = \frac{(\text{N intake} - (\text{faecal N} + \text{urine N}))}{\text{N intake}} \cdot 100$$

In these experiments the following diets were used:

1. Prestarter for baby pigs (Rød laktal)
2. 50% N from animal protein (skim milk powder) + 50% N from vegetable protein (oat kernels)
3. Only vegetable protein (50% N from soya bean meal + 50% N from oat kernels)

The N content in the 3 diets was kept constant throughout each experiment but at different levels, i.e., diet no. 1 contained about 4% N in dry matter, whereas the others contained about 2%. It was considered advisable to include different protein sources as well as different N levels, since some of the conflicting opinions might well be due to differences in technique.

2. Commercial prestarter as nitrogen source

The experimental animals comprised 4 male pigs of Danish Landrace × Gloucester Old Spot weaned at 13 days of age. Weaning was followed by a preliminary period of 6 days and thereafter 10 consecutive balance periods of 3 days each. This procedure enabled the comparison of results of pigs from 20 up to 53 days of age. In each of the 10 periods assays were made of apparent digestibility (AD) and retention coefficient (RC) of the nitrogen. As mentioned earlier, the 3 first periods were used to check the individual variation and balances had to be discontinued for one week while waiting for the resluts of the chemical analyses before the pigs could be classed in groups on the basis of these results.

The relationships between the age of the pigs and the AD and RC values respectively are shown in Figure 7. The following regression equations were obtained:

Equation 11:

$$AD\ (\%) = 87.80 + 0.08 \cdot \text{age (days)}$$
$$s = 1.92, \quad s_b = 0.03, \quad r = 0.42$$

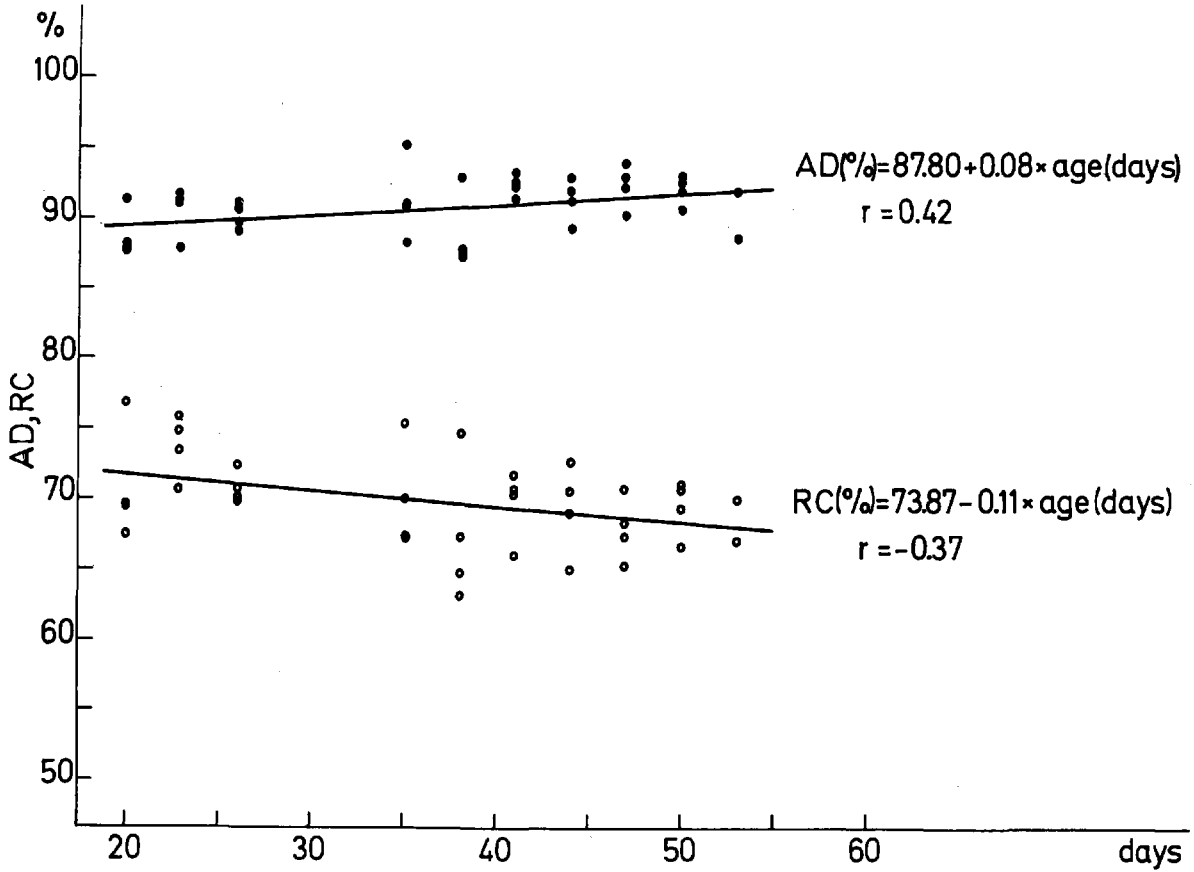

Figure 7. AD (apparent digestibility) and RC (retention coefficient) as related to the age of the pigs

Figur 7. TF (tilsyneladende fordøjelighed) og RC (retentionskoefficient) i relation til grisenes alder

Equation 12:

$$RC\ (\%) = 73.87 - 0.11 \cdot \text{age (days)}$$
$$s = 3.03, \quad s_b = 0.05, \quad r = -0.37$$

The regression coefficient for both AD and RC differs significantly from zero ($P < 0.05$). Thus the equations show that AD has a tendency to increase whereas RC has a tendency to decrease with increasing age of pigs. The results for AD agree with the findings of *Lloyd et al.* (1957), while the figures for RC appear to support the suggestions mentioned above.

Although the age of the animal does not appear to influence protein digestibility to any appreciable extent in the range studied, this might not be true in the case of the individual amino acids.

To investigate this, feed samples and the corresponding faeces samples of 3 periods were analysed for amino acids and the AD values calculated. The results are shown in Table 8.

Table 8. Apparent digestibility of individual amino acids in pigs of different ages

Tabel 8. Tilsyneladende fordøjelighed af de enkelte aminosyrer hos grise på forskellige alderstrin

Age of the pigs (days)	26		41		50	
	AD (%)	s	AD (%)	s	AD (%)	s
Lysine	87.7	1.2	87.0	0.4	87.1	1.0
Methionine	93.4	2.7	93.4	0.5	93.5	0.4
Cystine	88.1	1.0	90.4	0.6	89.7	0.5
Aspartic acid	87.5	1.5	90.4	1.3	90.3	0.9
Threonine	89.7	1.5	91.9	1.3	91.8	1.6
Serine	91.4	1.1	93.2	0.9	93.1	1.2
Glutamic acid	92.8	1.0	93.6	1.1	94.6	0.9
Glycine	81.4	2.3	85.5	2.6	86.2	3.9
Alanine	82.5	2.0	86.1	1.2	86.1	2.4
Valine	92.9	0.7	94.3	0.3	94.4	1.0
Isoleucine	89.2	1.5	91.5	1.1	92.1	1.6
Leucine	91.8	1.2	93.6	0.8	93.9	1.1
Tyrosine	91.8	1.3	93.1	1.2	93.2	1.5
Phenylalanine	89.2	1.9	92.3	1.5	91.4	1.6
Histidine	95.5	0.7	96.2	0.6	96.2	0.9
Arginine	86.0	1.4	89.4	1.0	89.0	1.3
Total N	90.1	0.8	92.5	0.7	92.1	1.1

It appears from Table 8 that the amino acids generally have lower AD values (about 2 units) when the pigs are 26 days old compared to 41 and 50 days. This is, however, in agreement with the AD values for total N

in the respective periods. Furthermore, it appears that the availability of the different amino acids within the same protein matter might differ, in this case up to 8–10 AD units. The standard deviation (s) of the AD values was generally low, suggesting that estimates of this kind can be made with a fairly high degree of accuracy. It can thus be concluded that there is no evidence to indicate that the age of the pigs influences the AD values of the individual amino acids differently from the AD values of total N.

3. Skim milk powder plus oat kernels as nitrogen sources

Since most of the protein in »Rød laktal« is of animal origin no conclusion can be made concerning the behaviour of animals fed diets containing a higher level of vegetable protein. To elucidate this problem an experiment was carried out in which 50% of the nitrogen in the diet was of vegetable origin. For this purpose oat kernels were employed together with skim milk powder. The experimental animals comprised 4 male pigs of Danish Landrace × Gloucester Old Spot, but AD and RC were in this experiment measured only in 7 three-day periods from 34 days up to 52 days of age. The procedure was identical to that employed in the previous experiment with the exception of the N concentration in the diet which was estimated to be approximately half of that used in the prestarter diet. The main reason for the lower N content was to prevent a suppression of the experimental data – especially the RC values – due to a high N concentration. Furthermore it was considered of interest to determine whether a low N content would lead to refusal of the diet by these very young pigs. The latter question could easily be answered, however, since there were no problems with regard to diet consumption.

Figure 8 depicts the relationship between the age of the pigs and the AD and RC values respectively when the pigs were fed a diet with 50% of the protein of vegetable origin.

The regression equations were calculated as:

Equation 13:

$$\text{AD (\%)} = 86.53 - 0.03 \cdot \text{age (days)}$$
$$s = 2.44, \quad s_b = 0.08, \quad r = -0.08$$

Equation 14:

$$\text{RC (\%)} = 53.05 - 0.003 \cdot \text{age (days)}$$
$$s = 2.81, \quad s_b = 0.09, \quad r = -0.01$$

In contrast to the previous experiment, the regression coefficient for these equations were not found to be significant at $P > 0.05$. Thus the ex-

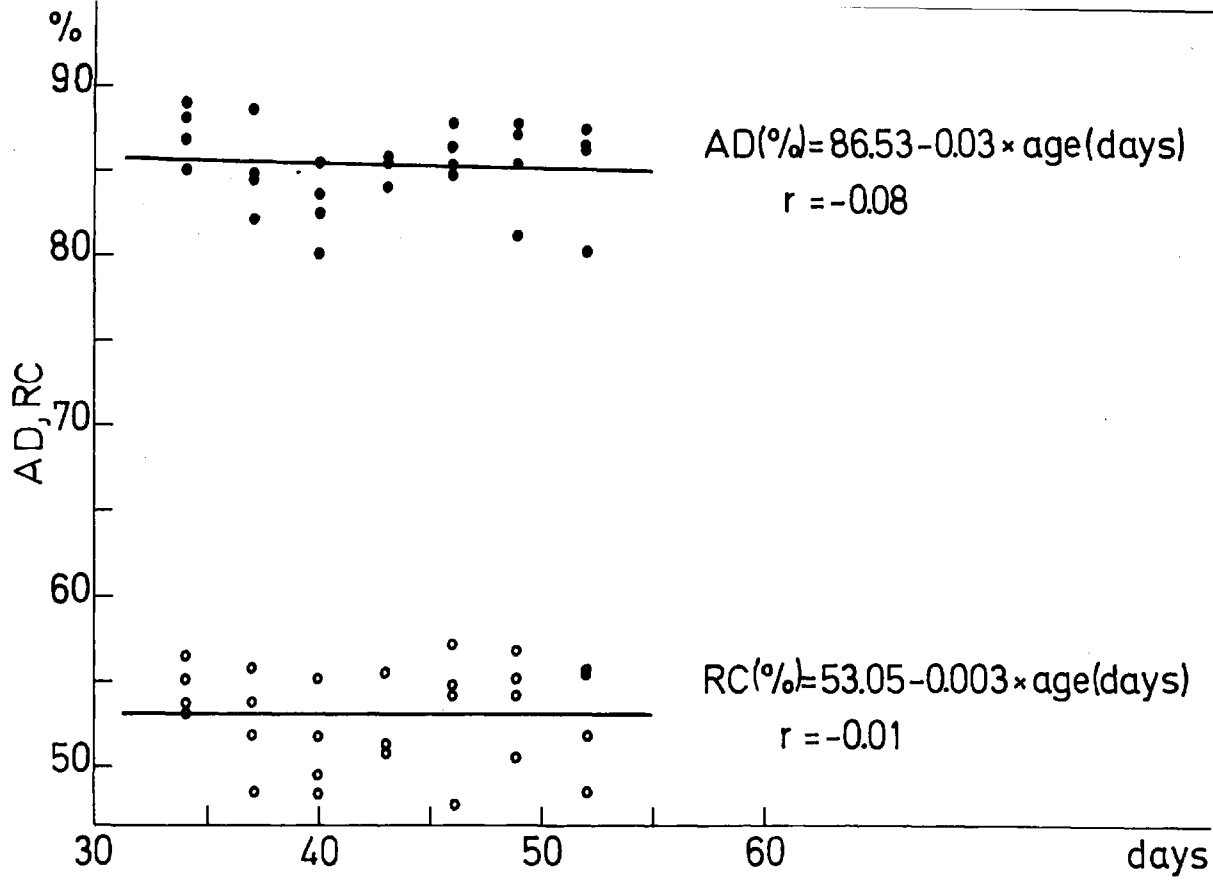

Figure 8. AD (apparent digestibility) and RC (retention coefficient) as related to the age of the pigs

Figur 8. TF (tilsyneladende fordøjelighed) og RC (retentionskoefficient) i relation til grisenes alder

periment provided no indication of any influence of age on the protein utilization.

It is of interest to note the much lower RC values obtained with this diet compared to those obtained with »Rød laktal«. This might be due to a poorer protein quality, but the main reason is surely the lower N content in this diet. As will be discussed later, metabolic N and endogenous N have a much greater influence on the AD and RC values when the N content is low.

4. Soya bean meal plus oat kernels as nitrogen sources

As most of the protein sources to be discussed later are of vegetable origin an experiment was carried out to investigate the influence of age on protein utilization when the pigs were fed entirely on vegetable protein. A mixture of soya bean meal and oat kernels was used as protein source and the N content was about 2% of dry matter. This experiment also comprised 4 pigs, but of pure Danish Landrace. The pigs were measured for 7 three-day periods from 34 up to 52 days of age.

In the following regression equations the relationship is shown between the age of the pigs and the AD and RC values respectively when the pigs were fed protein of this vegetable origin (see Figure 9):

Equation 15:

$$AD\ (\%) = 78.45 + 0.07 \cdot \text{age (days)}$$
$$s = 3.01,\quad s_b = 0.10,\quad r = 0.15$$

Equation 16:

$$RC\ (\%) = 60.45 - 0.14 \cdot \text{age (days)}$$
$$s = 2.39,\quad s_b = 0.08,\quad r = -0.34$$

The regression coefficients for both AD and RC do not differ significantly from zero at $P > 0.05$. This would appear to indicate that 30 days old pigs utilize vegetable protein just as effectively as 50 days old pigs.

From this work it can be seen that in one experiment the age of the pigs appeared to influence protein utilization, whereas in the other two experiments no significant effect of age was found. It should be emphasized that in the experiment where the age of the pigs appeared to affect the results the pigs were started at 20 days of age, while in the other experiments the pigs were started at an age of 34 days. This might explain the differences in the results of the experiments. In all following experiments the pigs were not started on the experimental diet until they were 30 days old.

As will be seen later, the results from these 3 experiments have been employed in estimating TD and BV of the respective diets and also in the comparisons with results obtained with rats fed the same diets.

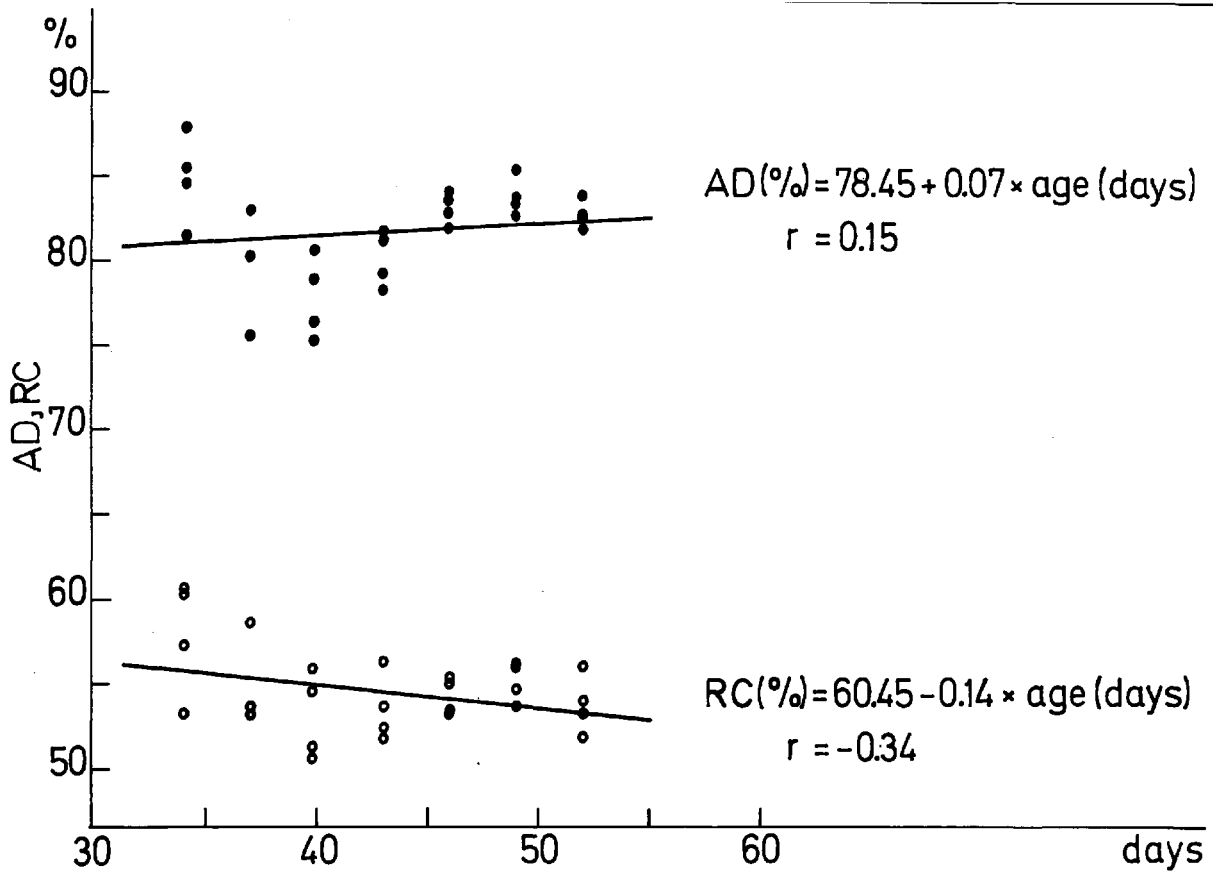

Figure 9. AD (apparent digestibility) and RC (retention coefficient) as related to the age of the pigs

Figur 9. TF (tilsyneladende fordøjelighed) og RC (retentionskoefficient) i relation til grisenes alder

CHAPTER V

A short discussion of methods for the estimation of amino acid availability

A. General discussion

Only limited results are to be found in the literature concerning the availability of amino acids in foods and feedstuffs. That the availability of the invidual amino acids might vary can be inferred from previous in vitro digestion studies (*Mitchell & Hamilton* 1929, *Jones & Gersdorff* 1933, *Melnick et al.* 1946) which indicate that amino acids are liberated from proteins at different rates characteristic of the amino acid or its linkage in the protein.

Both in vitro and in vivo methods have been used for estimating the availability of amino acids in proteins. The values obtained, however, differ considerably.

The in vitro methods are based either on comparisons of the rate at which amino nitrogen or free amino acids are released from proteins when incubated in vitro with proteolytic enzymes (*Riesen et al.* 1947, *Melnick & Oser* 1949, *Mauron* 1973) or on measurement of the percentage of free ϵ-amino groups of lysine in proteins by the fluorodinitrobenzene (FDNB) procedure (*Carpenter & Ellinger* 1955, *Carpenter* 1960). The former method provides a relative rather than a quantitative measure of amino acid availability; the latter is a quantitative method – but only for lysine. The FDNB method is useful for estimating the effects of heat processing, which lowers particularly the availability of lysine by causing binding of ϵ-amino groups.

The in vivo methods are based either on the measurement of the increase in faecal excretion of a particular amino acid after feeding a test protein (*Kuiken & Lyman* 1948), the ability of a protein of known amino acid composition to replace a specific amino acid in supporting growth or repletion of the animal (*Schweigert* 1948, *Schweigert & Guthneck* 1953, *Schweigert & Guthneck* 1954). in maintaining nitrogen balance in a mature subject (*Linkswiler et al.* 1958a, *Linkswiler et al.* 1958b, *Linkswiler et al.* 1960a, *Linkswiler et al.* 1960b) or determination of free amino acids in blood and muscle (*Pion* 1973, *Eggum* 1973).

The in vivo methods can be used to determine the availability of almost any amino acid. The interpretation of the estimate obtained will, however, depend upon the choice of method. When determined by the faecal analysis

method »availability« represents the amount of unabsorbed amino acid. It is thus a measure of digestibility applied to a specific amino acid and will depend upon the digestibility of the protein and the presence of enzyme resistant peptide bonds or enzyme inhibiting substances in the samples under investigation. The growth and nitrogen balance methods, on the other hand, assess not only digestibility, but also the efficiency of utilization of the absorbed amino acids.

In general, values obtained by the faecal analysis method are higher than those obtained by the growth method (*Guthneck et al.* 1953, *Gupta et al.* 1958, *Calhoun et al.* 1960), particularly for poorly balanced proteins such as those of maize. Values as low as 50% for the availability of lysine from maize have been obtained using the growth method (*Gupta et al.* 1958) as compared to 89% by the faecal analysis method (*de Muelenaere & Feldman* 1960). For isoleucine in zein, a value of 30% was obtained using the growth method (*Deshpande et al.* 1957); by the faecal analysis method the isoleucine of maize was estimated to be 90% available (*de Muelenaere & Feldman* 1960). In contrast, in other experiments in which growth has been used as the criterion of availability, values well in excess of 100% have been obtained (*Lushbough et al.* 1957, *Ousterhout et al.* 1959).

The values obtained by the growth method will be influenced not only by the amount of amino acid lost in the faeces but also by inefficient utilization due to delayed release of amino acids which may result in lower values than are obtained by the faecal analysis method. However, growth is also influenced by amino acid balance, protein level, type of carbohydrate and the calorie/protein ratio of the diet. Variation in any of these factors may affect values for availability obtained by the growth method; some of the abnormally low or high values obtained by this method are probably the result of inadequate control over these factors (*de Muelenaere et al.* 1967).

Although results obtained by the faecal analysis method are less subject to the influence of the above factors, other criteria may influence the accuracy of this method. As will be discussed, the method has been criticized on account of the possible influence of microbial activity in the intestine. The synthesis or destruction of amino acids by the intestinal microflora would result in lower or higher availability values respectively. Several methods have been devised to measure the availability of amino acids but unfortunately these methods do not measure the same thing. For a summary of the various methods proposed the reader is referred to the review of *Harper & de Muelenaere* (1963). The present discussion will be confined chiefly to the results obtained with the faecal analysis method.

The faecal analysis method is analogous to the determination of true digestibility of the total protein. It consists of measuring the amount of amino acid ingested in the diet, the amount excreted in the faeces and the so-called

metabolic losses in the faeces. The latter is estimated from the amount of amino acid excreted by an animal fed a protein-free diet or a diet with completely digestible protein (ether-extracted, freeze-dried egg protein) and is adjusted for differences in feed intake in the same way as in the determination of the true digestibility of a protein. Availability is calculated according to equation 17:

Equation 17:

$$\text{Availability} = \frac{\text{amino acid intake} - \left(\text{faecal amino acid excretion} - \text{metabolic amino acid excretion}\right)}{\text{amino acid intake}} \cdot 100$$

The faecal analysis method was developed by *Kuiken & Lyman* (1948) who employed it to determine the availability of ten indispensable amino acids in several proteins. Availability as determined by the faecal analysis method should provide a measure of the quantity of amino acid that is released and absorbed during digestion. As indicated by *Harper & de Muelenaere* (1963), this method should have particular merit in determining which amino acids are excreted in indigestible or insoluble residues from poorly digestible proteins.

The nitrogen excreted in the faeces is derived from four sources: (a) unabsorbed food (feed) nitrogen, (b) unabsorbed residues of gastrointestinal secretions, (c) cell of the mucosa of the intestinal lining and (d) the intestinal flora. The gastro-intestinal tract is in a dynamic state, receiving various fluids rich in digestive enzyme systems containing nitrogen and accumulating cellular debris, waste products and materials that are not absorbed or utilized by the body.

It should also be mentioned that nitrogenous compounds other than protein are secreted into the gastro-intestinal tract in the digestive juices. The most important of these is urea which is attacked by microorganisms, thereby releasing ammonia which is reabsorbed by the body and transformed to urea again by the liver (*Gitler* 1964). *Nasset* (1956) presented evidence that the dynamic state of the intestinal tract can alter the dietary pattern of amino acids. He also suggested that the bacterial flora may contribute to such a change.

Using two different methods, *Gupta & Elvehjem* (1957) determined the availability of tryptophan in several feedstuffs fed to weaned rats. The first method involved the determination of unavailable tryptophan in the faeces, while the second made use of a comparison between the growth of rats fed the test material and the growth of animals receiving rations containing graded levels of tryptophan. Despite the criticism that bacterial activity in the gastro-

intestinal tract renders the faecal analysis method unsuitable for availability determination, no significant difference in tryptophan availability was found between these two methods.

It is difficult to assess whether a lack of complete absorbtion affects amino acids selectively or is more general. Several authors (*Kuiken & Lyman* 1948, *Kuiken* 1952, *Gupta & Elvehjem* 1957, *Gupta et al.* 1958, *Borchers* 1962) have investigated this aspect by assaying the recoveries of individual amino acids in the faeces. As previously mentioned, this approach is complicated by the admixture of amino acids from the diet with amino acids derived from the endogenous secretion of the intestine wall and by the transformation of amino acids by bacteria in the lower parts of the intestine. *Munro* (1964), however, considered the availability of amino acids in foods to be reasonably complete, since the biological value and chemical score of proteins are generally closely correlated.

Armstrong & Mitchell (1955), using pigs, found that increasing the intake of protein resulted in a linear increase in the amount of nitrogen in the faeces. In experiments with dogs *Allison & Bird* (1964) found that the rate of increase of nitrogen eliminated in the faeces with increasing protein intake was greater for soya bean meal, which was moderately digestible, than for highly digestible casein. These results were verified by *Carlson & Bayley* (1970) in experiments with piglets. These observations thus indicate that the digestibility and amino acid composition of dietary protein can influence amino acid output in the faeces, in spite of the great dilution of exogenous dietary protein with endogenous secretion into the alimentary tract observed by *Nasset & Ju* (1961). However, it appears that in rats the endogenous secretions are extensively reabsorbed by the time they reach the end of the ileum even though the extent of autodigestion and reabsorption of the endogenous protein is reduced by the presence of exogenous protein in the digesta (*Snook & Meyer* 1964). Thus the level of inclusion of the highly digestible casein would have less influence on amino acid output in the faeces than the moderately digestible soya bean meal.

de Muelenaere & Feldman (1960) determined the availability of lysine, isoleucine, threonine and methionine in rats fed three samples of maize grown under different levels of fertilizer application. They found no significant differences between samples; the availability was approximately 90% for lysine and threonine, 90% for isoleucine and 95% for methionine. It was concluded that the method used to determine availability in this study, although sometimes criticized on account of potential changes which might be brought about by microbial activity in the intestinal tract, is the only reliable method for the determination of amino acid availability in poorly balanced proteins.

In an investigation by *Kuiken & Lyman* (1948) the essential amino acids in roast beef were found to be completely available to the rat, the availability

ranging from 99.2 to 100.7%. Similarly the total nitrogen of the meat protein was also found to be completely available. It is of interest that roasting the beef did not decrease the availability of the amino acids. Quite divergent results were obtained with a sample of cotton seed flour (*Kuiken* 1952) in which marked variation in the availability of the individual amino acids was observed. Although 93% of the arginine was available to the rat, only about 65% of the lysine was available. The values for the other amino acids varied within these two extremes. *Kuiken* (1952), however, considered it unlikely that the high percentage of lysine found in the faeces from the animals fed cotton seed flour was due to bacterial synthesis. In experiments with groundnut flour and wheat *Kuiken & Lyman* (1948) reported availability values ranging from 94.8 to 99.5 for groundnut flour and from 92.8 to 98.8 for wheat.

Dammers (1964) carried out extensive experiments with adult pigs and showed that individual amino acids from the same feedstuff might have different TD values. This was particularly pronounced in the case of heat-treated nutrients such as skim milk powder. Toasting decreased the TD of lysine from 100.6 to 66.2%, whereas methionine showed a lesser decrease (from 100.6 to 90.8). *Poppe et al.* (1969c) also demonstrated varying availabilities of amino acids in skim milk powder given to chickens. Lysine and threonine had very low TD values (72 and 77% respectively) while phenylalanine was digested with 96%. *Hermann & Kreienbring* (1969) found with chickens that the apparent digestibility of the amino acids might differ from one amino acid to another. This was pronounced for barley and maize and the values for lysine were low.

The above results indicate that in certain cases information on the amino acid content of foods and feedstuffs is inadequate in the evaluation of protein quality unless availability data are also obtained. While the availability of nitrogen and individual amino acids were found to be closely correlated in roast beef, groundnut meal and wheat (*Kuiken & Lyman* 1948), there were marked exceptions in the case of cotton seed meal (*Kuiken* 1952). The results for cotten seed meal indicate the extent to which total amino acid data might be misleading.

B. The quantitative calculation of metabolic amino acids

In the present work the faecal analysis method is employed to calculate the true digestibility of individual amino acids. As is seen from Equation 17 this necessitates a quantitative calculation of amino acids of metabolic origin. As metabolic protein (nitrogen) is directly related to dry matter ingested, a similar relationship must apply for metabolic amino acids. Rats excrete

2.04 mg metabolic N per gram dry matter consumed, while pigs excrete metabolic N according to Equation 7 : mg N in faeces = 127.26 + 0.49 · g dry matter consumed. Having the amount of metabolic protein excreted together with the amino acid composition of this protein, the quantitative amounts of individual amino acids of metabolic origin can easily be calculated. The amino acid composition of metabolic protein of rats and pigs are shown in Table 9. With exception of figures for proline, tryptophan and ammonia, the figures are identical with those given in Table 7.

Table 9. Amino acid composition of metabolic protein of rats and pigs respectively

Tabel 9. Aminosyresammensætning i stofskifteprotein hos henholdsvis rotter og grise

Animal species	Rats (g/16 g N)	Pigs (g/16 g N)
Lysine	5.88	5.65
Methionine	2.00	2.03
Cystine	1.92	1.61
Aspartic acid	8.86	8.34
Threonine	4.61	4.38
Serine	7.71	7.01
Glutamic acid	10.31	10.66
Proline	3.21	–
Glycine	4.11	4.48
Alanine	5.03	5.64
Valine	4.55	4.82
Isoleucine	3.75	3.97
Leucine	5.45	6.07
Tyrosine	3.82	3.44
Phenylalanine	3.92	5.09
Histidine	2.21	2.09
Arginine	4.18	4.19
Tryptophan	1.13	–
Ammonia	1.68	–

In the present work different expressions for % absorbed amino acids are employed, namely true digestibility, availability and absorbability. These terms are used synonymously and thus no attempt will be made to differentiate between them.

As mentioned above, the faecal analysis method has been criticized on account of the possible influence of microbial activity in the intestine. In the following chapter the significance of the microflora in the gastro-intestinal tract on protein metabolism will be discussed.

CHAPTER VI

The significance of the microflora in the gastro-intestinal tract on protein metabolism

A. General discussion

Investigations of the type reported here are often subject to the criticism that microbial action in the gastro-intestinal tract may affect protein metabolism. Complete evidence that errors due to this cause are quantitatively insignificant will require further investigation. In this chapter the question as to whether microbial activity is sufficient to modify the biological value of the diet is considered.

The presence of a population of microorganisms in the alimentary tract of conventional animals has given rise to considerable speculation as to the extent to which microbial activity influences the course of digestion and metabolism of proteins and as to whether the results are beneficial or detrimental to the host. One possibility is that amino acids released during digestion might be absorbed by microorganisms and thus rendered unavailable to the host (*Fauconneau & Michel* 1970). A second possibility proposed by *Nesheim & Carpenter* (1967) is that proteins of dietary or endogenous origin escaping digestion in the small intestine might be catabolized by microbial enzymes in the lower gut with subsequent absorption of the end products. If these end products were amino acids or simple nitrogenous compounds (e. g. ammonia) from which non-essential amino acids could be synthesized, they might be of benefit to the host. Alternatively they might be of no nutritional value, as for example, biologically unavailable peptides; excess ammonia would also be valueless (*Fauconneau & Michel* 1970).

Columbus et al. (1958) found in experiments with growing rats that the faecal content of bacterial origin was negligible when the animals were fed a nitrogen-free diet. Furthermore the weight of the experimental animals did not affect the relative amount of microbial nitrogen. However, several experiments have shown that the intestinal flora can degrade amino acids, amides, urea, etc. Thus *Michel* (1966) reported that the caecal flora of the pig is capable of degrading all amino acids by decarboxylation or deamination. Similiar results have been obtained from studies of the faecal output of amines in calves (*Michel & Mathieu* 1967). These studies showed that few amino acids were degraded in the healthy calf, whereas in the animal with diarrhoea faecal excretion of free amino acids and their degradation products was much increased. Faecal cadaverine as a percentage of lysine eaten was only 0.4% in normal

calves while faecal putrescine as a percentage of arginine eaten was 0.8%. According to *Michel et al.* (1964) both nutritional and environmental factors determine the type of intestinal flora present and consequently its metabolic role. In the case of the suckling pig, faecal excretion of amines arising from microbial degradation did not occur. However, the excretion of amines increased after weaning and was maximal when diarrhoea occurred *(Michel et al.* 1964).

Various investigations of the action of the intestinal flora on faecal N excretion have shown conflicting results. *Luckey* (1963) found levels of excretion of the same magnitude in both axenic and control animals. However, *Levenson & Tennant* (1963) reported higher levels of faecal N in the faeces of germ-free rats, similar results being obtained by *Miller* (1967) and *Salter & Coates* (1971) with germ-free chicks. In contrast to these observations, *Harmon et al.* (1968) observed that axenic rats given a sterile liquid diet had a much lower output of faecal N at zero protein intake than control animals. *Harmon et al.* (1968) presented evidence that these differences in faecal output in the axenic versus control groups may reflect the amount and nature of the diet ingested. Although emphasis has generally been placed on changes in the morphology of the small intestine in germ-free animals *(Gordon & Wostmann* 1960, *Abrams et al. 1963, Gordon & Bruckner-Kardoss* 1961a, *Gordon & Bruckner-Kardoss* 1961b), it is nevertheless in the caecum that the action of the flora appears to be most important.

It is thought that in chicks the bacteria of the caecum play a role in the proteolysis of poorly digestible proteins such as raw soya bean *(Nitsan* 1965) and deteriorated fish concentrates *(Payne et al.* 1968).

Valle-Riestra & Barnes (1970) suggested that bacterial proteolysis in conventional rats may increase the apparent digestibility of protein damaged by heat, whereas *Giovanetti et al.* (1970) showed that the prevention of coprophagy had no significant effect on the availability of amino acids to rats in a pure wheat diet.

However, *Loesche* (1968a, 1968b) fed germ-free rats on a protein-free diet and found that the protein content of the caecum was similar to that found in germ-free rats receiving dietary protein. According to *Fauconneau & Michel* (1970), this may indicate that the excessive amount of organic nitrogen in the caecum of the germ-free animal is derived from endogenous protein that is not digested in the lower small intestine in absence of a bacterial flora.

The results of *Loesche* (1968b) appear to support this view, the majority of the proteins found in caecal contents of germ-free rats being derived from the host and not from the diet. Additional experiments showed that conventional animals reabsorb 80 to 90% of the nitrogen-containing compounds found in the contents of the distal half of the small intestine, whereas these compounds were not reabsorbed in the distal half of the germ-free small intestine.

Thus it appears that the germ-free rat cannot efficiently degrade and reabsorb the endogenous protein which are shed into its gastro-intestinal tract. This is possibly the reason for the enlarged caecum which can average up to 20% of the body weight of certain germ-free rats *(Loesche* 1968a). In conventional animals this part of the intestinal tract is about 1–2% of the body weight.

In experiments with germ-free and conventional chicks *Salter & Coates* (1971) found rather surprisingly a general similarity in both presence and absence of microorganisms. The distribution of C^{14} and N in the upper part of the alimentary tract was much the same in germ-free and conventional birds and it was only in the lower intestinal contents and excreta that any significant effect of environment was observed. This was true both for good and poor quality proteins. There was, however, no evidence that protein availability to the bird was improved by microbial action in the intestine. This is in contrast to findings reported by *Nitsan* (1965) and *Payne et al.* (1968).

Salter & Coates (1971) suggested that the small differences indicated between the two environments in the absorption of amino acids were unlikely to have been of importance in the protein nutrition of the bird. Furthermore they suggest that some of the ammonia released by microbial action could, if absorbed, has been utilized by the bird for synthesis of non-essential amino acids. Again such an effect, if it existed, would not have been detectable on a good diet but might become apparent with a low protein intake. In the trials by *Salter & Coates* (1971) the total N measured in the excreta of conventional chicks was less than in the corresponding samples from germ-free birds, although the difference was not statistically significant.

It is obvious that there is a substantial synthesis of vitamins by the intestinal flora *(Jayne-Williams & Coates* 1969) but there is little other quantitative information available. The situation is made more difficult to interpret by the marked morphological and physiological differences between germ-free and contaminated animals. It is thus difficult to differentiate between the contributions that might be made by the microorganisms and the secondary physiological changes *(Lewis & Swan* 1971).

According to *Cranwell* (1968) fermentation takes place in all regions of the alimentary tract of the pig and commences within the first week of life. The substrates fermented and the end products of fermentation depend on a number of factors, the most important being the age of the pig, the type of the diet and the region of the intestine. Carbohydrate, protein and fat in the diet may all undergo some form of microbial attack, but fermentation of carbohydrate is the most extensive and the most likely to be of economic importance (*Cranwell* 1968). The influence of coprophagy on absorption has been studied by *Giovannetti et al.* (1970) who found that the prevention of coprophagy had no significant effect on the availability of amino acids in

a pure wheat diet consumed by male rats weighing 80 or 300 g. Prevention of coprophagy in rats consuming a supplemented wheat diet or pure wheat had no significant effect on weight gain over a period of 35 days or on nitrogen balances at the two different stages of growth. There were no significant differences in the average availability of each amino acid between weight groups. This indicates that a possible effect of microflora on TD does not increase with age. Similar results were reported by *Just Nielsen* (1968, 1971) who found that the apparent digestibility of total N and individual amino acids in diets for bacon pigs remained unchanged from 20 to 90 kg body weight. *Giovannetti et al.* (1970) found the average availability of each of the amino acids studied in wheat to be above 90% in both weight groups. Glutamic acid and proline were most readily available in both groups. Aspartic acid followed by alanine and lysine were the least available to the younger rats, while lysine followed by alanine and aspartic acid were the least available to the older rats. *Nesheim* (1965), however, pointed out the discrepancy between absorbed and available amino acids, especially in poorly digested or heat-damaged proteins, and advanced the hypothesis that protein and peptides leaving the small intestine were fermented in such a way that nitrogen was absorbed in a form such as ammonia. This would give an erroneous picture of the amino acids actually absorbed by the animal. *Nesheim* (1965) suggested, however, that with well digested proteins this may not be a problem, since relatively little nitrogen reaches the lower intestinal tract.

It can be seen from this discussion that widely different opinions exist with regard to the significance of the microflora in the gastro-intestinal tract on protein metabolism. However, some of the disagreement might be due to differences in the physiological condition of the experimental animals. This applies particularly to comparisons between conventional and germ-free animals since the physiological state of conventional animals might differ significantly from one laboratory to another. In general, however, the microflora does appear to affect the course of digestion and metabolism of proteins. However, the possible significance of this microbial activity is still a matter of conjecture. Attempts have been made to determine whether antibiotics have any detectable effect on organisms of the intestinal tract, in addition to the suppression of those causing disease. If this proves to be the case antibiotics may well be a valuable tool in obtaining information on the nutritional significance of the microflora.

B. Action of antibiotics on the bacterial flora

Modifications in the metabolism of intestinal organisms can be obtained by the addition to the diet of small doses of antibiotics (10 or 20 parts per million). Chlortetracycline (*Larson & Hill* 1955) and oxytetracycline and sodi-

um acrylate (*Michel et al.* 1964) are known to inhibit the formation of nitrogenous bases in the intestine of the young pig. *Michel* (1956) and *Michel & Francois* (1956) reported that the microflora of the intestinal contents of pigs were very active in the decarboxylation of amino acids. Aspartic acid and glutamic acid were readily decarboxylated. Arginine, histidine, alanine and tryptophan were also attacked, while lysine, ornithine and methionine were rarely affected. Decarboxylation of tryptophan was not consistently inhibited by chlortetracycline, whereas decarboxylation of aspartic acid and arginine was markedly reduced. Furthermore *Michel & Francois* (1956) showed that microbial decarboxylation of the individual amino acids is not consistently inhibited by chlortetracycline. Thus a supplement of chlortetracycline to a diet should affect the TD value of the individual amino acids to different degrees if the microbial decarboxylation is pronounced.

In agreement with the work of *Michel & Francois* (1956), *Melnykovych & Johansson* (1955) reported a reduction of amine formation in the intestine of rats fed chlortetracycline. *Larson & Hill* (1960) showed in experiments with young pigs that the metabolic activities of the microflora of the ileum were markedly reduced when small amounts of chlortetracycline were imposed. They obtained a greater amount and variety of amines from pigs receiving the basal diet without chlortetracycline supplementation. Amines produced by decarboxylases from Escherichia coli were found to be common to both groups, but were present in smaller amounts in the group fed chlortetracycline.

Fauconneau & Michel (1970) stated that the characteristics of animals receiving antibiotics are similar to those of germ-free animals, particularly with regard to the reduction in thickness of the small intestine and the increase in volume of the caecum.

Kuiken (1952) fed 2% succinylsulphathiazole in a ration containing cotton seed meal and measured the amino acid availability by using the faecal analysis method (*Kuiken & Lyman* 1948, *Harper & de Muelenaere* 1963). There were no significant differences in the availability coefficients irrespective of whether or not the rats were fed succinylsulphathiazole. *Kuiken* suggested that the effect from the intestinal bacteria on the amino acid availability was negligible. The same conclusion was reached by *Dammers* (1964) in experiments with adult pigs, a supplement with sulphathiazole to meat and bone scraps having no significant effect on amino acid availability.

Assuming that the microflora does have a significant effect on protein metabolism in the intestine and keeping in mind that the extent of microbial destruction of individual amino acids may differ considerably, differences in digestibility coefficients of individual amino acids might be expected.

1. Present investigations

In an attempt to elucidate some of the problems discussed above, experiments were carried out with rats fed barley supplemented with chlortetracycli-

ne or sulphathiazole. The results obtained for the TD of total nitrogen (Kjeldahl N) and for the individual amino acids were compared with the corresponding results from rats fed unsupplemented barley. In addition results from one group of specific pathogen-free rats (SPF) fed unsupplemented barley were compared with the results from the other groups. The amino acid determinations were performed on freeze-dried samples of the faecal material.

Although none of the three experimental groups were germ-free, it is reasonable to assume a reduction in microorganisms in the alimentary tract of these groups compared to the unsupplemented group. Assuming the microflora to have a marked influence on the TD values, this should enable us to determine possible differences. The results are given in Table 10.

Table 10. True digestibility of the individual amino acids and total nitrogen in barley measured on rats with or without added chlortetracycline or sulphathiazole
Tabel 10. Sandfordøjelighed af de enkelte aminosyrer og totalkvælstof i byg målt på rotter med og uden tilsætning af henholdsvis klortetracykline og sulfatiazol

Experimental rats	Conventional	SPF	Conventional	Conventional
Supplement	None	None	Chlortetracycline (20 ppm)	Sulphathiazole (2% in diet)
TD	(%)	(%)	(%)	(%)
Lysine	76.7	78.1	81.3	80.5
Methionine	80.8	82.6	84.6	84.1
Cystine	92.2	90.0	90.6	90.6
Aspartic acid	78.8	78.4	81.2	80.4
Threonine	81.5	79.1	83.1	80.0
Serine	99.7	100.0	100.0	99.3
Glutamic acid	93.9	95.9	95.4	96.8
Glycine	79.4	80.3	82.7	78.9
Alanine	77.9	78.8	82.1	83.0
Valine	89.4	89.7	91.6	92.5
Isoleucine	84.8	85.5	87.9	89.7
Leucine	87.6	88.7	90.0	91.6
Tyrosine	87.9	84.6	91.4	89.6
Phenylalanine	88.6	88.2	87.4	90.2
Histidine	90.6	91.8	93.9	90.9
Arginine	89.7	91.6	91.4	91.0
Tryptophan	90.3	90.6	92.6	91.3
Total nitrogen	85.1	85.6	87.5	87.2

As expected the TD values obtained on rats fed chlortetracycline or sulphathiazole are slightly higher than those in the other groups (*Eggum* 1972), although this difference is no more than 1–3% in most cases. It should be emphasized that the TD values in general show the same trend from group to group. In the case of lysine and glutamic acid for example, lysine can

be seen to have low TD values in all cases while glutamic acid has high values. Furthermore about half of the amino acids show lower TD values than that obtained for total nitrogen while the remainder show slightly higher values. This situation provides a basis for a discussion of the influence of the microflora on the values obtained.

Assuming that the microflora is active in the decarboxylation of amino acids (*Michel* 1956, *Michel & Francois* 1956), higher TD values for individual amino acids than for total nitrogen would be expected if microbial activity is of significance. The above authors found that aspartic acid and glutamic acid were readily decarboxylated, but that this was markedly reduced in the presence of chlortetracyline. There is no real evidence in Table 10 to support this theory; aspartic acid shows a low TD value, i. e., a relatively high content of this amino acid is present in the faeces. The high TD obtained for glutamic acid is probably due to the high content of this amino acid in the readily digested prolamin fraction in barley as will be discussed later. Decarboxylated amino acids may be incorporated into microbial protein and defaecated and hence a readily decarboxylated amino acid need not necessarily give high TD values. Amino acids such as lysine and methionine which are rarely attacked (*Michel & Francois* 1956) show, however, the same response to supplements of chlortetracycline or sulphathiazole as the other amino acids. The low TD for lysine could indicate a high content of microbial protein in the faeces since this protein is rich in lysine (*Weller* 1957, *Mason & Palmer* 1971). However, this does not apply for the low TD for methionine as microbial protein is low in sulphur-bearing amino acids.

A study of the results in Table 10 obtained with rats shows no obvious indication of a significant influence due to the microflora on the TD values obtained and thus supports the statement made by *Dammers* (1964), namely that the microflora does not appear to have any appreciable influence on the true digestibility of the individual amino acids in pigs.

C. The influence of the nitrogen-free matter in the diet on the TD values of the individual amino acids

According to *Michel et al.* (1964) both nutritional and environmental factors determine the type of flora present in the intestine and hence their metabolic role. To elucidate this aspect experiments were conducted with casein, barley, and casein + barley and TD values for total N and for the individual amino acids were calculated. Due to the high N concentration of casein, the greatest part of the N-free fraction in this diet was derived from autoclaved potato starch, whereas in the barley groups almost all of the N-free fraction came

from the barley itself. In the group fed casein + barley the situation is intermediate.

The results of these experiments are shown in Table 11. The values for barley in the last group were corrected for undigested amino acids in the faeces originating from casein by using the TD values given for this protein. Owing to the high TD of casein, however, the amount of amino acids in faeces derived from this protein is negligible and thus only small corrections were required.

Table 11. True digestibility of the individual amino acids of casein and barley measured on rats when fed alone and of barley when fed together with casein
Tabel 11. Sand fordøjelighed af de enkelte aminosyrer i kasein og byg når de fodres hver for sig, samt af byg når der fodres sammen med kasein, målt i forsøg med rotter

TD	Casein fed alone (%)	Barley fed alone (%)	Barley fed with casein (%)
Lysine	99.4	76.2	77.1
Methionine	99.1	81.8	80.3
Cystine	94.6	89.6	90.3
Aspartic acid	97.9	77.1	74.5
Threonine	97.9	80.2	78.9
Serine	98.1	91.2	89.6
Glutamic acid	96.5	94.1	95.5
Proline	97.8	92.3	93.6
Glycine	98.8	77.8	76.8
Alanine	99.7	78.7	76.1
Valine	98.4	85.4	84.1
Isoleucine	95.4	83.5	82.5
Leucine	98.7	86.8	88.7
Tyrosine	99.2	88.5	90.1
Phenylalanine	98.2	88.8	90.4
Histidine	99.9	89.8	90.7
Arginine	99.3	86.0	87.9
Tryptophan	98.5	87.7	86.3
Ammonia	99.1	87.0	87.9
Total N	98.8	86.1	87.4

The TD values of the individual amino acids appear to be independent of the carbohydrate source (Table 11). There are no distinct differences in the TD values for barley amino acids regardless of whether barley is fed alone or together with casein, providing a further indication that the microflora does not significantly influence TD values.

D. The influence of storage on amino acid composition of rat and pig faeces

According to *Erbersdobler* (1971) the microflora present in faeces samples may possibly transform some of the protein to microbial protein and ammonia. As the rat faeces in the present experiments were freeze-dried and generally stored for a few days before the amino acid analyses were conducted, changes in faeces protein due to the microflora might have occurred, giving rise to incorrect values. To elucidate this problem faeces samples from two different rats fed barley were analysed for amino acids before and after storage for 10 days in closed plastic boxes at room temperature (approximately 22° C). N content and pH were also determined. The results are listed in Table 12.

Table 12. Amino acid composition in freeze-dried rat faeces before and after storage for 10 days at 22° C.

Tabel 12. Aminosyresammensætning i frysetørret rottegødning før og efter lagring i 10 dage ved 22° C.

Rat no.	54	54	55	55
Stored (days)	0	10	0	10
Amino acids	(g/16 g N)	(g/16 g N)	(g/16 g N)	(g/16 g N)
Lysine	5.88	5.81	5.86	5.84
Methionine	2.08	2.11	1.98	1.93
Cystine	1.67	1.73	1.70	1.72
Aspartic acid	9.04	8.98	9.15	9.04
Threonine	4.45	4.60	4.60	4.74
Serine	3.68	3.78	3.83	3.92
Glutamic acid	10.68	10.91	11.10	11.21
Proline	4.61	4.80	4.87	4.81
Glycine	4.84	4.91	5.02	5.21
Alanine	5.26	5.28	5.27	5.38
Valine	4.85	4.85	4.79	4.90
Isoleucine	3.88	3.94	4.03	4.01
Leucine	5.60	5.65	6.08	5.98
Tyrosine	3.36	3.34	3.35	3.75
Phenylalanine	4.57	4.78	4.77	5.00
Histidine	1.78	1.68	1.85	1.91
Arginine	4.69	4.81	4.80	5.21
Tryptophan	0.93	0.94	0.96	0.96
Ammonia	1.75	1.68	1.67	1.62
N in % of dry matter	2.21	2.22	2.02	2.01
pH	5.97	5.91	6.08	6.00

None of the amino acids in Table 12 indicate any significant change in the protein composition of rat faeces after storage in 10 days at room temperature. This also applies to N content and pH. It can thus be concluded that

the microbial activity in freeze-dried rat faeces does not change the amino acid composition to any great extent.

As the situation might be different if the faeces are stored with the original water content, faeces samples from two pigs fed on different rations were collected and stored as such in closed plastic boxes and analysed for amino acids. The pigs weighed approximately 50 kg.

The first analysis was conducted immediately after defaecation and the second after storage at 6° C for 7 days, while the third analysis was carried out after an additional storage period of 7 days at room temperature. The results are given in Table 13.

Table 13. Amino acid composition in pig faeces stored at different temperatures

Tabel 13. Aminosyresammensætning i grisegødning opbevaret ved forskellige temperaturer

Pig no.	1			2		
Stored (days)	0	7	14	0	7	14
Temperature (°C)	–	6	6 and 22	–	6	6 and 22
Amino acids	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)
Lysine	5.31	5.19	5.34	6.58	6.61	6.49
Methionine	1.82	1.77	1.71	2.10	2.09	1.93
Cystine	1.11	1.23	1.14	1.30	1.28	1.24
Aspartic acid	8.38	8.44	8.10	8.66	8.63	7.90
Threonine	4.37	4.40	4.29	4.63	4.46	4.35
Serine	3.89	3.93	3.81	3.83	3.89	3.44
Glutamic acid	12.44	11.97	11.91	12.75	12.92	10.33
Proline	4.71	4.68	4.63	4.07	4.19	3.78
Glycine	4.59	4.66	5.07	4.97	4.98	5.02
Alanine	5.67	5.77	6.31	7.14	7.09	7.22
Valine	5.33	5.54	5.83	5.75	5.59	5.54
Isoleucine	4.51	4.48	4.67	4.16	4.04	4.34
Leucine	6.91	6.95	7.18	7.19	7.45	8.05
Tyrosine	3.57	3.81	3.77	4.08	3.96	3.52
Phenylalanine	5.29	5.37	5.24	5.03	5.33	5.13
Histidine	1.65	1.68	1.50	1.94	2.15	1.84
Arginine	3.98	3.75	3.30	4.01	4.00	3.83
Tryptophan	1.07	1.05	1.04	1.18	1.23	1.07
Ammonia	2.69	2.97	3.10	2.41	2.66	2.63
N (%)	1.033	1.032	1.051	0.956	0.962	0.996
Dry matter (%)	28.10	27.99	27.19	23.08	22.98	22.33
pH	6.22	5.99	5.82	6.29	5.79	5.50

The amino acid composition of untreated pig faeces shows little change when stored in closed plastic boxes at 6° C for 7 days (Table 13). Further storage for 7 days at room temperature, however, showed changes in certain

of the component amino acids, although there was no agreement between the two samples. The ammonia content increased slightly during storage and this, together with the decrease in pH, indicates significant microbial activity. The decrease in pH is probably due to the production af lactic acid (*Bønsdorff Petersen* 1969).

On the basis of the results shown in Table 12 and 13 it can be concluded that storage of faeces samples for a few days does not result in any appreciable change in the amino acid composition – particularly if the storage temperature is low.

E. The influence of dietary proteins on amino acid composition of faeces

Amino acids excreted with the faeces are exogenous as well as endogenous and thus it might be difficult to detect differences in amino acid composition of faeces samples despite the fact that the dietary proteins are of different origin. However, this is not found to be the case. Table 14 lists the amino acid composition of faeces originating from rats fed different protein sources.

Table 14. Amino acid composition of faeces collected from rats fed different proteins (average values of 5 rats per group)

Tabel 14. Aminosyresammensætning i gødning fra rotter fodret med forskellige proteinkilder (gens. værdier af 5 rotter pr. hold)

Protein fed	Barley		Groundnut meal		Fish meal	
	(g/16gN)	(s)	(g/16gN)	(s)	(g/16gN)	(s)
Lysine	5.77	0.21	5.38	0.09	5.34	0.08
Methionine	2.14	0.09	2.06	0.08	1.86	0.10
Cystine	1.80	0.12	1.57	0.09	1.43	0.04
Aspartic acid	9.23	0.16	8.96	0.39	7.82	0.15
Threonine	4.56	0.10	4.48	0.12	3.67	0.05
Serine	3.82	0.24	5.74	0.20	3.00	0.11
Glutamic acid	11.13	0.25	13.93	0.31	8.82	0.31
Proline	4.29	0.22	4.02	0.28	2.75	0.08
Glycine	4.75	0.20	4.67	0.18	4.07	0.08
Alanine	5.48	0.21	4.90	0.27	4.57	0.20
Valine	4.99	0.26	5.17	0.16	3.99	0.06
Isoleucine	4.16	0.23	4.69	0.17	3.46	0.15
Leucine	6.09	0.40	5.73	0.23	5.01	0.20
Tyrosine	4.20	0.35	3.87	0.29	2.93	0.13
Phenylalanine	4.76	0.10	3.74	0.23	3.27	0.12
Histidine	1.79	0.16	1.82	0.05	1.92	0.08
Arginine	4.15	0.17	3.95	0.12	3.17	0.10
Tryptophan	1.06	0.06	1.19	0.07	0.99	0.05
Ammonia	2.07	0.17	1.85	0.05	1.78	0.14

The faeces samples concerned are from rats fed on cereal protein, oil cake protein and fish protein respectively.

The amino acid composition of faeces can thus be seen to depend on the dietary protein. Furthermore it appears that the standard deviation (s) is independent of both protein source and amino acid. This situation provides a further indication that the microflora does not influence the results significantly.

In view of the extremely large number of microorganisms in the gastro-intestinal tract, even in monogastric animals, the microflora can obviously not be completely neglected in protein metabolism. However, from the work reported here it is tempting to conclude with the findings of *Fauconneau & Michel* (1970): »Even if, in the case of normal digestion, the overall function of the flora on metabolism of dietary nitrogen seems rather limited, and is more important in the case of the metabolism of endogenous nitrogen, nevertheless, the picture can be quite different in the case of malabsorption from various causes«.

F. A check of the sensitivity of the faecal analysis method in estimating the true digestibility of individual amino acids

As mentioned earlier protein is labile to heat treatment and of the amino acids lysine is particularly sensitive. In order to determine possible effects on lysine in heat-treated fish meal rat experiments were conducted with differently treated meals (*Gaardbo Thomsen* 1973) supplemented with a lysine deficient protein source (gluten). The diets contained 50% N from fish meal and gluten respectively. If the availability of lysine in the fish meals is affected

Table 15. Available lysine plus TD, BV and NPU of differently treated fish meals supplemented with gluten

Tabel 15. Tilgængelig lysin samt SF, BV og NPU i forskelligt behandlet fiskemel suppleret med gluten

Fish meal no.	1	2	3	4
Temp. °C	0	121	121	121
Hours	0	3	6	9
	(%)	(%)	(%)	(%)
Available lysine in fish meal	78.3	72.8	67.0	63.1
True digestibility of fish meal + gluten	94.8	91.6	87.1	84.3
Biological value of fish meal + gluten	79.9	73.9	71.0	69.0
Net protein utilization of fish meal + gluten	75.8	67.7	61.9	58.2

a reduction in the BV would be expected with increasing heat treatments. As these fish meals are identical with those described by *Gaardbo Thomsen* (1973), data for available lysine are quoted from the latter report. It should be noted that no correction factor is used, i.e., the data for % available lysine given in Table 15 are data actually measured in the respective fish meal samples. Together with data for available lysine (*Carpenter* 1960), expressed as % of total lysine, values are given for TD, BV and NPU obtained in experiments with rats.

It can be seen from Table 15 that available lysine decreases almost linearly from 78.3 to 63.1 in the unsupplemented fish meals 1 to 4. If lysine is the limiting amino acid in these diets (fish meal + gluten) a corresponding decrease in BV would be expected. However, a considerably smaller decrease in BV (from 79.9 to 69.0) was observed. This is undoubtedly due to the fact that available lysine from the untreated part (gluten) of the diets constitutes an increasing part from sample 1 to 4, i.e., available lysine in the diets is not reduced to the same extent as the data for the fish meals would indicate. Furthermore the true digestibility of total N was also found to decrease with increasing heat treatments. This would indicate that not only is lysine reduced, but also several other amino acids if not all. In order to provide further information on this problem a second experiment was carried out in which the fish meals constituted the sole nitrogen source and the true digestibility of all amino acids was estimated by means of the faecal analysis method.

In general the amino acid composition can be seen to be hardly affected by the present heat treatments. Significant effects are found only for lysine and cystine, lysine decreasing from 7.60 to 7.18 and cystine from 0.85 to 0.73 g/16gN when samples 1 and 4 are compared. Similar results were obtained in experiments with fish meal carried out by *Mason & Weidner* (1964).

The TD for total N is reduced from 90.5 to 77.5 from sample 1 to 4, i.e., a decrease of 13.0 units, while the corresponding value for lysine is 14.1 (92.2 to 78.1). If lysine is assumed to be the limiting amino acid in sample 4 a corresponding reduction would be expected in BV.

This is also seen to be the case, BV decreasing by 13.4 units from 80.3 to 66.9. If methionine is assumed to be the limiting amino acid the decrease in BV should correspond to the decrease in availability of this amino acid. However, the availability of methionine is affected to the same degree as lysine and thus comparison with this amino acid does not change the situation, the TD of methionine decreasing from 91.8 to 77.3, i.e., 14.5 units.

By comparing the data in Table 15 for available lysine measured according to *Carpenter's* procedure, this value can be seen to decrease by 15.2 units from 78.3 to 63.1, i.e., of the same magnitude as found in the in vivo determinations. Furthermore the availability of all amino acids is reduced due to heat treatment, most of them by 10–15 units. Thus the data do not indicate lysine

Table 16. AAC plus TD, BV and NPU in differently heat-treated fish meals determined in experiments with rats

Tabel 16. ASS samt SF, BV og NPU i forskelligt varmebehandlet fiskemel i forsøg med rotter

Fish meal no.	1		2		3		4	
Temp. °C	0		121		121		121	
Hours	0		3		6		9	
Criteria	AAC (g/16gN)	TD (%)	AAC (g/16gN)	TD (%)	AAC (g/16gN)	TD (%)	AAC (g/16gN)	TD (%)
Lysine	7.60	92.2	7.39	85.2	7.17	81.4	7.18	78.1
Methionine	2.59	91.8	2.66	87.3	2.40	79.6	2.58	77.3
Cystine	0.85	88.7	0.80	83.0	0.74	78.8	0.73	74.7
Aspartic acid	9.41	91.5	9.37	84.6	9.86	79.1	9.86	77.3
Threonine	4.34	92.6	4.41	89.0	4.40	83.0	4.18	79.3
Serine	3.98	97.4	3.88	94.0	3.87	88.4	3.70	85.0
Glutamic acid	13.53	93.2	13.56	90.0	13.77	84.7	13.65	82.9
Proline	4.29	93.4	3.92	86.0	3.88	81.4	4.07	81.6
Glycine	6.17	91.7	6.10	87.1	6.19	83.0	6.21	81.0
Alanine	6.06	91.6	6.03	87.7	6.06	82.8	6.23	81.3
Valine	5.27	92.0	5.22	87.2	5.33	81.4	5.43	81.5
Isoleucine	4.40	91.3	4.49	87.6	4.42	81.4	4.44	79.9
Leucine	7.51	92.5	7.55	88.5	7.47	82.1	7.45	80.7
Tyrosine	2.33	90.8	2.86	86.3	2.94	82.1	2.94	76.7
Phenylalanine	3.79	92.0	3.88	87.4	3.89	79.3	3.80	79.1
Histidine	2.38	91.9	2.38	89.6	2.28	82.7	2.26	80.6
Arginine	5.77	95.1	5.71	92.1	5.57	88.6	5.79	85.5
Tryptophan	1.45	92.7	1.10	81.8	1.19	75.5	1.17	73.1
Ammonia	1.21	85.5	1.23	80.2	1.21	75.8	1.29	72.6
N in % of dry matter	12.93		13.06		12.99		12.78	
Protein value expressed as a percentage:								
TD	90.5		84.7		80.5		77.5	
BV	80.3		70.6		67.1		66.9	
NPU	72.7		59.8		54.0		51.9	
Body weight (g)	91.6		77.8		71.2		71.8	

to be more heat sensitive than the other amino acids. However, it must be taken into consideration that total lysine is reduced by approximately 6% (7.60 to 7.18) and that fish meal is also very low in carbohydrates. The average body weights at the end of the experiments show a difference of 19.8 g from group 1 to 4. The corresponding reduction in NPU is 20.8 units. This shows a sound agreement between these two criteria and indicates that the faecal analysis method is both sensitive and reliable in the in vivo calculation of TD.

When considering the value of the faecal analysis method in estimating the biological availability of the individual amino acids in feedstuffs, the extensive nature of the task should be borne in mind. This aspect has been extensively discussed by *Harper & de Muelenaere* (1963) in relation to the methods available.

An accurate appraisal of the nutritional value of proteins will demand the development and perfection of methods of estimating amino acid availability. However, on the basis of the above discussion and the present experimental results, the faecal analysis method was considered to be reliable and suitable for this purpose. This method was therefore adopted in the following work. TD values of the individual amino acids were determined in several feedstuffs in experiments with rats and pigs and these values set in relation to the biological value.

CHAPTER VII

Availability of individual amino acids and protein quality in fifteen diets as determined in experiments with rats and pigs

A. General discussion

In order to contribute to the knowledge of amino acid availability in feedstuffs, TD values for individual amino acids were determined in the present investigations. Fifteen different protein sources were evaluated on both rats and pigs and comparisons are made between these two. In addition to TD values for individual amino acids, the protein value is also expressed as:

1. Amino acid composition (AAC)
2. Available amino acids (AAA)
3. True digestibility of total protein (TD)
4. Biological value (BV)
5. Net protein utilization (NPU)
6. Utilizable nitrogen (UN)

It can be assumed that the amino acids supplied in free form are completely available to the organism. In view of the fact that proteins may not be completely digested, the availability of the amino acids in proteins might well influence amino acid requirements and render the results obtained with artificial diets containing pure amino acids inapplicable to natural diets. Animal feeding trials clearly demonstrate that the unavailability of lysine is an important practical problem (*Carpenter et al.* 1962) and that arginine, tryptophan and cystine may also be destroyed or rendered unavailable (*Liener* 1958). However, in studies of the availability of the valine, threonine and isoleucine of maize, *Linkswiler et al.* (1958a, 1958b, 1960a, 1960b) did not find the requirements of these amino acids to be higher when supplied as cereal compared with the free forms.

In the present investigation six different grain proteins, six concentrated feedstuffs of animal or vegetable origin and three mixtures were evaluated. The protein sources tested are listed in Table 17.

As can be seen from Table 17, twelve of the samples contain protein from only one protein source, while three samples are made up of two or more protein components.

Table 17. List of feedstuffs evaluated in experiments with rats and pigs
Tabel 17. Oversigt over fodermidler vurderet i forsøg med rotter og grise

1. Barley	9. Meat and bone scraps
2. Oats	10. Soya bean meal
3. Wheat	11. Groundnut meal
4. Rye	12. Sunflower seed meal
5. Maize	13. Skim milk powder + dehulled oats
6. Sorghum	14. Soya bean meal + dehulled oats
7. Casein	15. Prestarter for baby pigs (Rød laktal)
8. Fish meal	

With rats the nitrogen concentration in the experimental diets was kept constant, each animal receiving 150 mg N and 10 g dry matter per animal per day for all diets. With pigs, however, the samples were fed at different nitrogen concentrations. This was due to the fact that in the initial experiment the baby pigs were fed a prestarter (sample 15) and it was considered desirable to maintain the original N concentration (3.84% N of dry matter). Since no difficulties were experienced in this experiment, the N concentration in the 6 subsequent samples (7–12) was reduced to 3.0% N of dry matter. With some of these diets a reduction in appetite was observed. This did not appear to be related to the lower N concentration, but rather to be a question of protein quality. The N concentration was therefore further reduced (2.0% N) in the following two experiments (samples 13 and 14). These experiments were carried through without difficulties in spite of the fact that 50% of the nitrogen was derived from grain protein. This prompted the evaluation of diets consisting solely of grain protein. The protein concentration in these diets was adjusted to 1.5%N of dry matter.

As will be seen, variation in the N concentration of the diets considerably influences BV values. The validity of a comparison of BV values obtained at different N contents in the diets is therefore questionable. Consequently a direct comparison between BV values obtained on rats and pigs respectively in this series can only be made on the grain diets. However, as the primary intention with these experiments was to measure the true digestibility of the individual amino acids and of total nitrogen, this disadvantage with regard to BV values had to be accepted. As TD is independent of the N concentration in the diet it was preferred to work with only one nitrogen-carrying substance in the diet at a time. Thus the grain components could be fed alone.

In the following discussion the 15 diets will be treated separately as this will give the best survey of the material. Furthermore, AD values from the literature will not be included in the present discussion since a comparison of AD and TD values is extremely difficult, particularly if the N concentration in the experimental diet is not stated (Figure 13).

B. Experiments with barley

Barley is known to be low in lysine and will normally show a positive response to a lysine supplement (*Madsen et al.* 1969, *Eggum* 1970b). Threonine seems to be the second limiting amino acid (*Atkinson & Carpenter* 1968), while the content of sulphur-bearing amino acids is relatively good. However, considerable variation in protein quality from one barley lot to another is observed (*Nehring & Bock* 1961). This might be explained by differences in growing conditions (*Bengtsson & Eggum* 1969, *Schiller* 1971). An increase in nitrogen in barley due to increased N fertilization generally gives rise to a decrease in lysine when expressed as g/16 g N (*Eggum* 1970b, *Thomke* 1970, *Schiller* 1971). As lysine is the principle limiting amino acid in barley, a change in this particular amino acid will necessarily influence the biological criteria. This has been demonstrated by *Brune et al.* (1968), *Eggum* (1970b) and *Schiller* (1971). An increase in nitrogen causes an increase in TD whereas BV decreases. These conditions might explain the conflicting results obtained with barley.

Hennig (1957) obtained a TD of 91.8 and a BV of 82.9 in experiments with adult pigs, while *Dammers* (1964) found a TD of 82.9. In work with rats *Wünsche & Bock* (1965) obtained a TD of 89.3 and a BV of 74.8.

Very few results for the TD of individual amino acids are available. However, *Dammers* (1964) found in experiments with adult pigs that TD for the individual amino acids in barley differed considerably, ranging from 74.1 for methionine to 93.3 for cystine. Lysine had a TD of 89.0, aspartic acid, glycine and alanine had TD values in the lower part of the range, while histidine, arginine, glutamic acid and serine were in the upper part; the TD for total N was 82.9. In experiments with rats *Poppe et al.* (1969a) measured the TD of total N, lysine and methionine in a number of feedstuffs. The values for barley were 80.2, 78.3 and 86.4 for total N, lysine and methionine respectively. In experiments with pigs *Poppe et al.* (1969b) found a TD for lysine of 56.8, while valine was absorbed with 84.7%.

It can thus be seen from these references that the biological criteria in barley differ significantly. In the following table the results of the present investigation with rats and pigs are listed.

Considerable variation can be seen in the TD of the individual amino acids, ranging from values above to values below TD for total nitrogen. Certain of these differences were found to be significant. The low values for lysine are of particular interest as lysine is the limiting amino acid in grain protein. The TD values for aspartic acid, glycine and alanine are lower than for total nitrogen, whereas glutamic acid, histidine and arginine are considerably higher. With the exception of lysine, these data are very much in line with the findings of *Dammers* (1964). Morevoer, the TD values for rats and pigs can be seen to follow the same trend, i.e., low values obtained with rats generally corre-

Table 18. AAC, AAA plus TD, BV, NPU and UN of barley determined in experiments with rats and baby pigs
Tabel 18. ASS, TAS samt SF, BV, NPU og UN i byg bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	3.69	76.0**)	3.1	2.80	72.3***)	3.6	2.67
Methionine	1.82	80.5	6.7	1.47	77.5*)	6.2	1.41
Cystine	2.30	91.4**)	3.4	2.10	89.3***)	3.3	2.05
Aspartic acid	6.99	78.1	7.7	5.46	77.8*)	5.4	5.44
Threonine	3.60	80.1	5.0	2.88	77.8*)	5.0	2.80
Serine	4.16	96.4***)	4.4	4.01	88.1**)	4.0	3.66
Glutamic acid	25.06	91.4**)	2.3	22.90	90.4***)	2.3	22.65
Glycine	4.46	78.8	4.7	3.51	79.1	4.9	3.53
Alanine	4.59	77.5	6.1	3.56	75.5**)	6.6	3.47
Valine	5.33	84.0	5.5	4.48	82.5	4.3	4.40
Isoleucine	3.68	80.5	6.0	2.96	79.2	4.0	2.91
Leucine	7.11	85.0	4.5	6.04	84.1	3.4	5.98
Tyrosine	3.71	81.5	6.4	3.02	83.8	5.9	3.11
Phenylalanine	4.91	83.4	2.5	4.09	80.7	4.7	3.96
Histidine	2.23	87.4	5.1	1.95	87.6**)	3.1	1.95
Arginine	5.38	85.8	4.6	4.62	88.9***)	3.1	4.78
N in % of dry matter (barley)		1.62			1.62		
N in % of dry matter (diet)		1.51			1.54		
Protein value expressed as a percentage: TD		82.0	2.3		82.4	4.3	
BV		71.8	2.3		80.8	7.8	
NPU		58.9	2.7		66.7	7.8	
UN		0.95	0.04		1.08	0.13	

*) $p < 0.05$; **) $p < 0.01$; ***) $p < 0.001$
(significantly different from TD of total N)

sponded to low values with pigs, etc. The TD for total nitrogen was the same in both rats and pigs, 82.0% and 82.4% respectively. BV, however, was higher in the experiments with pigs. Due to the low N content in barley the UN was relatively low (approximately one).

The low TD value of lysine is probably due to differences in lysine concentration and TD in the different protein fractions described by *Osborne* (1895) (albumin, globulin, glutelin, prolamin, non-protein N). The lysine content is highest in the aleuron layer and lowest in the endosperm (*Schiller* 1971). Since the endosperm possesses the highest digestibility (*Munck* 1964) and the lowest lysine content, a lower TD for lysine will be obtained when estimated on total N. Similarly, higher TD values are obtained for glutamic acid which is abundant in the endosperm. It is of interest to note that *Munck* (1971)

obtained the highest concentrations for lysine, aspartic acid and alanine in the non-extractable part of barley protein (according to *Osborn* 1895). This corresponds with the low TD values obtained for these amino acids in present work.

C. Experiments with oats

Oats is the cereal which generally shows the highest lysine and cystine contents, while the concentration of the other amino acids is very much the same as for barley. In contrast to barley, however, the protein quality of oats seems to be much more resistant to ecological factors. This has been shown in experiments by *Bengtsson & Eggum* (1969) in which the influence of place of growth and N fertilization was examined. Analogous to barley the protein content in oats increased with increasing N fertilization, but in contrast to barley g lysine and methionine + cystine/16 g N were independent of N fertilization. Furthermore the place of growth did not affect the protein quality in oats whereas this was the case with barley. These differences in response might be due to the much lower prolamin content in oats (10–15%) compared to barley (30–35%). Glutelin is almost the only reserve protein in oats and consequently this protein fraction will increase with increasing N fertilization. In barley, however, the prolamin fraction which is poorer in lysine will increase.

In the experiments of *Bengtsson & Eggum* (1969) considerably less variation was found in the TD and BV values for oats (2 varieties) than for barley. In oats TD ranged from 85 to 90 and BV from 67 to 72, whereas the corresponding ranges for barley were 76 to 90 and 70 to 78 respectively. However, in experiments with rats fed on several varieties of oats grown in different years, *Nehring & Bock* (1961) found an average TD value of 85.1 but with a range from 83.0 to 91.2. BV of the corresponding samples had an average value of 68.8 and a range from 63.0 to 75.2. This work indicates considerable variation in the protein quality of the different varieties of oats. *Wünsche & Bock* (1965) measured a TD as high as 93.7% and a BV of 70.3, while *Mitchell* (1954) and *Lang & Schoen* (1952) found a BV of 65.0. *Tang et al.* (1958) found in growth studies with rats fed oat diets supplemented with amino acids that lysine was the principle limiting amino acid. Supplements of lysine, methionine and threonine were required to obtain a rapid growth rate. Mean availability of nitrogen was measured to 86%, while lysine and methionine were found to be 84 and 85% available respectively. Threonine, however, had an availability of only 72%.

In experiments with pigs *Brune et al.* (1968) did not find N fertilization to have any negative effect on the quality of oat protein; BV was 58.9 with low N fertilization but 61.9 with higher N fertilization. The corresponding

TD values were 74.9 and 71.9 respectively. *Hennig* (1957), however, measured a TD of only 34.6 but a BV of 81.5.

Results of the present investigation with oats are shown in Table 19.

Table 19. AAC, AAA plus TD, BV, NPU and UN of oats determined in experiments with rats and baby pigs

Tabel 19. ASS, TAS samt SF, BV, NPU og UN i havre bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	4.03	79.6*)	1.3	3.21	73.0**)	5.0	2.94
Methionine	1.77	84.3	3.6	1.49	76.6	5.2	1.36
Cystine	3.03	89.0	2.7	2.70	90.1***)	2.1	2.71
Aspartic acid	8.66	83.1	3.0	7.20	78.3	3.5	6.78
Threonine	3.63	80.4	4.4	2.92	76.9	4.3	2.79
Serine	4.98	94.7***)	0.3	4.72	88.1***)	2.4	4.39
Glutamic acid	22.10	89.2*)	1.4	19.71	88.9***)	2.0	19.65
Glycine	5.45	85.6	2.9	4.67	80.5	2.7	4.39
Alanine	4.91	82.0	3.9	4.03	74.0**)	4.1	3.63
Valine	5.12	84.6	3.4	4.33	79.2	3.6	4.06
Isoleucine	3.98	83.2	3.2	3.31	77.8	4.2	3.10
Leucine	7.07	84.8	2.4	6.00	82.1**)	2.6	5.80
Tyrosine	3.53	86.5	2.8	3.05	78.9	5.9	2.79
Phenylalanine	4.87	84.0	5.2	4.09	78.7	4.2	3.83
Histidine	2.21	89.9*)	2.1	1.99	85.0***)	2.5	1.88
Arginine	6.07	88.2	2.0	5.35	87.3***)	2.1	5.30
N in % of dry matter (oats)		1.72			1.72		
N in % of dry matter (diet)		1.49			1.50		
Protein value expressed as a percentage: TD		84.1	2.6		78.6	2.9	
BV		70.4	4.3		76.4	5.3	
NPU		59.1	2.6		60.0	4.3	
UN		1.02	0.05		1.03	0.08	

It would appear from Table 19 that rats digest oat protein more efficiently than pigs, with TD values of 84.1 and 78.6 respectively. However, the TD in pigs seems to be rather low, the TD obtained with rats agreeing with the referred data. As was also the case with barley, BV for oats was higher when measured on pigs than on rats, 76.4 and 70.4 respectively. TD values for the individual amino acids are very much the same as for barley. The low values for lysine should be emphasized. Due to this the amount of available lysine in oat protein is quite low.

D. Experiments with wheat

It is well known that the protein quality in wheat is inferior to that in barley and oats. This is primarily due to the very low lysine and threonine contents. Moreover, the concentration of sulphur-bearing amino acids is lower than in the other cereals. As for barley, the protein quality of wheat appears to be negatively affected by N fertilization (*Lindner* 1963, *Larsen & Dissing Nielsen* 1966). Increasing nitrogen supplies cause an increase in glutamic acid and proline but a simultaneous decrease in lysine and arginine when expressed in g/16 g N. Investigations by *Nehring* (1963) showed that wheat with high protein content possessed a lower biological value than wheat lower in protein. The following regression equation was found between BV and protein %: BV = 87.60 – 1.61 · % protein. *Brune et al.* (1968) found in experiments with pigs that the protein quality in wheat was considerably reduced with increasing N fertilization. Similarly *Eggum* (1970a) found protein-rich Mexican wheat varieties to possess lower BV values than a common Danish wheat variety, although TD was high in the protein-rich varieties and indicated high TD values for the component amino acids.

In experiments with rats *Mitchell* (1954) measured a BV of 67. *Lang & Schoen* (1952) obtained similar results, whereas *Nehring & Bock* (1961) obtained a value of 69. Furthermore *Nehring & Bock* (1961) obtained an average value for TD of winter wheat from Austria ranging from 88.8 to 93.4. The corresponding BV values averaged 60.0, and ranged from 56.3 to 62.2. In the case of spring wheat, *Nehring & Bock* (1961) found a TD of 86.9 and a BV of 68.3.

Kuiken & Lyman (1948) showed in experiments with rats the availability of all essential amino acids in wheat to be extremely high and to range from 92.2 to 98.8. de *Muelenaere & Feldman* (1960) found availability coefficients of 89.5, 92.8, 88.7 and 95.3 for lysine, isoleucine, threonine and methionine respectively. *Calhoun et al.* (1960) estimated lysine availability by performance and used percentage of added lysine to a basal diet as a standard. By this method the availability of lysine in wheat, flour, bread and gluten was found to be 78, 80, 83 and 80% respectively. In work with rats fed with wheat by-products *Olsen et al.* (1968) found the absorbability of the individual amino acids to vary considerably. It should be emphasized that the trend in the TD values of these products was very much the same as for the present results for barley, oats and wheat. This would in general suggest low values for lysine, alanine and aspartic acid and high values for glumatic acid, histidine and arginine. The results of the present experiments with wheat are listed in Table 20.

The TD values can be seen to be higher in wheat than in barley and oats, whereas BV is considerably lower due to the low lysine content in wheat. TD for total N is very much the same in both rats and pigs, 91.8 and 89.6

Table 20. AAC, AAA plus TD, BV, NPU and UN of wheat determined in experiments with rats and baby pigs

Tabel 20. ASS, TAS samt SF, BV, NPU og UN i hvede bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	2.55	79.2**)	4.9	2.02	84.1***)	3.0	2.14
Methionine	1.82	86.1	8.9	1.57	88.6	4.8	1.61
Cystine	1.81	95.1**)	1.0	1.72	93.9	2.5	1.70
Aspartic acid	5.26	83.8*)	4.1	4.41	87.1*)	6.1	4.58
Threonine	3.02	88.6	3.4	2.62	88.4	5.1	2.67
Serine	4.73	100.0***)	2.0	4.73	96.5**)	2.9	4.56
Glutamic acid	35.77	99.0***)	1.0	35.41	97.4***)	1.1	34.84
Glycine	4.19	86.2	2.4	3.61	89.7	4.0	3.76
Alanine	3.77	83.0*)	4.5	3.13	86.5*)	6.0	3.26
Valine	4.58	90.3	2.2	4.14	90.8	3.7	4.16
Isoleucine	3.38	88.2	2.3	2.98	90.3	4.9	3.05
Leucine	6.79	91.5	1.8	6.21	93.2	2.8	6.33
Tyrosine	3.14	88.9	3.4	2.79	92.5	4.2	2.90
Phenylalanine	4.41	89.8	4.0	3.96	92.0	3.9	4.06
Histidine	2.29	96.1**)	2.2	2.20	95.9**)	2.6	2.20
Arginine	4.65	92.8	1.7	4.32	95.0*)	3.0	4.42
N in % of dry matter (wheat)		2.02			2.02		
N in % of dry matter (diet)		1.52			1.48		
Protein value expressed as a percentage: TD		89.6	3.7		91.8	3.3	
BV		59.0	2.9		71.2	4.8	
NPU		52.9	4.1		65.2	3.3	
UN		1.07	0.09		1.32	0.07	

respectively. BV is highest in pigs with 71.2 compared to 59.0 in rats. The values obtained in rats appear to be in line with results from the literature.

TD values for the individual amino acids are similar in rats and pigs. As for the other grain proteins, TD of lysine in wheat is also significantly lower than TD of total N. The same found *Poppe & Meier* (1971a) in experiments with birds. In addition to lysine, aspartic acid and alanine also have significantly lower TD values than total N, whereas glutamic acid, histidine and arginine have significantly higher values. Since the TD values are all relatively high the differences between total and available amino acids in wheat are not as great as in barley and oats.

E. Experiments with rye

The use of rye as animal feed has not always given favourable results, and little work has been carried out on protein quality in this species (*Clausen*

1961a). No real differences are found in the amino acid composition of rye and other cereal proteins and thus the unfavourable results obtained by feeding rye cannot be explained in terms of severe amino acid deficiencies.

A high protein quality in rye has been reported. *Nehring & Bock* (1961) found a TD of 80.5 and a BV of 78.4 in experiments with rats, while *Wünsche & Bock* (1965) obtained even higher values with TD of 88.8 and BV of 84.2. As shown in Table 21 the present investigation also gave relatively high values for the biological criteria.

Table 21. AAC, AAA plus TD, BV, NPU and UN of rye determined in experiments with rats and baby pigs

Tabel 21. ASS, TAS samt SF, BV, NPU og UN i rug bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	3.67	65.3***)	2.8	2.40	72.2***)	3.3	2.65
Methionine	1.68	75.0	3.7	1.26	73.9***)	2.8	1.24
Cystine	1.98	83.0*)	3.5	1.64	86.3**)	3.7	1.71
Aspartic acid	7.25	73.1	5.3	5.30	76.9	5.5	5.58
Threonine	3.31	71.9	5.9	2.38	74.4**)	6.6	2.46
Serine	4.21	91.6***)	4.0	3.86	88.1**)	4.9	3.71
Glutamic acid	23.62	91.1***)	2.3	21.52	92.2***)	1.8	21.78
Glycine	4.63	73.8	5.2	3.42	78.2	5.4	3.62
Alanine	4.53	71.7	5.5	3.25	73.3*)	5.6	3.32
Valine	4.57	78.8	4.3	3.60	78.0	5.6	3.56
Isoleucine	3.11	74.4	4.6	2.31	73.6**)	6.6	2.29
Leucine	5.91	79.4	3.7	4.69	81.2	4.2	4.80
Tyrosine	2.92	71.1	3.9	2.08	79.1	4.7	2.31
Phenylalanine	4.42	75.2	5.4	3.32	79.8	4.2	3.53
Histidine	2.32	84.3*)	4.9	1.96	88.8***)	3.8	2.06
Arginine	5.65	83.6*)	2.6	4.72	88.5***)	3.7	5.00
N in % of dry matter (rye)		1.46			1.46		
N in % of dry matter (diet)		1.42			1.41		
Protein value expressed as a percentage: TD		77.0	4.6		80.9	4.4	
BV		76.7	1.2		79.7	6.3	
NPU		59.0	3.8		64.5	5.8	
UN		0.86	0.06		0.94	0.09	

The TD values were found to be lower in rye than in the other cereals, particularly in the case of rats. TD for total N was 77.0 when measured with rats and 80.9 when measured with pigs. As in the other cereals BV was higher in the experiment with pigs than with rats, 79.7 and 76.7 respectively. TD for the individual amino acids varied considerably, the lowest value

being that for lysine. With the exception of methionine which shows low TD values, the trend in the TD values of rye is similar to the situation found in the other cereals studied.

The results of the present investigation and those of other workers show that protein quality in rye is very similar to that in barley and oats. The conflicting experiences with rye might be due to the relatively high content of 5-alkylrecorcinol (*Holmberg & Ohlson* 1968). This substance may have a negative influence when rye is fed over long periods, although no harmful effects are found in short balance periods.

F. Experiments with maize

The low lysine and tryptophan contents of maize are well known and contribute to a relatively low biological value. The contents of sulphur-containing amino acids and threonine, however, are relatively high (Table 22). The amino acid composition depends, however, on the protein level in maize (*Sauberlich et al.* 1953), protein-rich maize containing a higher proportion of zein than maize with less protein.

Hennig (1957) reported a TD of 99.9 and BV of 40.7 in work with pigs. In experiments with rats *Lang & Schoen* (1952) found a BV of 60.0, while *Mitchell* (1954) reported 62.0 and *Nehring & Bock* (1961) 68.3 and a TD of 88.0. The new maize mutant Opaque-2 (*Mertz et al.* 1964) is known to possess a higher protein content than normal hybrid maize, but also has a higher lysine and tryptophan content. In experiments with Opaque-2 on rats (*Eggum* 1969a) a TD of 93.4 and BV of 69.9 were obtained in spite of an N content in dry matter of 2.13% in the original material. With regard to the availability of the individual amino acids, *de Muelenaere & Feldman* (1960) found TD coefficients of 89.5, 92.8, 88.7 and 95.3 for lysine, isoleucine, threonine and methionine respectively. In experiments with birds *McNab & Shannon* (1971) found that in general the basic amino acids were quite highly digestible in maize germ meal. The present experiment was carried out with a normal hybrid maize.

The TD of maize protein can be seen from Table 22 to be approximately 90 (87.6 for rats and 90.2 for pigs), while the corresponding BV values are 58.1 and 72.6. These figures are almost identical with the results obtained with wheat.

The TD values of the individual amino acids in maize differ from the other cereals discussed in showing considerably less variation. None of the amino acids possessed TD values significantly lower than total N. This was also true in the case of lysine, a fact of considerable importance. It is of interest that similar results were obtained by *Poppe & Meier* (1971b) in experiments with birds.

Table 22. AAC, AAA plus TD, BV, NPU and UN of maize determined in experiments with rats and baby pigs

Tabel 22. ASS, TAS samt SF, BV, NPU og UN i majs bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	2.73	84.6	5.0	2.31	89.3	5.7	2.44
Methionine	2.35	90.4	2.2	2.12	93.6*)	2.4	2.20
Cystine	2.27	90.7	1.4	2.06	91.4	3.2	2.07
Aspartic acid	7.19	88.3	4.6	6.35	91.1	2.6	6.55
Threonine	4.00	87.1	3.4	3.48	89.8	3.1	3.59
Serine	4.98	95.0***)	2.1	4.73	96.8***)	2.5	4.82
Glutamic acid	17.46	91.9**)	1.6	16.05	92.2	2.8	16.10
Glycine	4.04	85.9	2.9	3.47	89.6	3.4	3.62
Alanine	8.19	90.0	2.7	7.37	93.0**)	1.9	7.62
Valine	5.01	89.7	2.5	4.49	89.9	2.6	4.50
Isoleucine	3.77	88.4	2.3	3.33	88.5	2.9	3.34
Leucine	10.60	91.0	1.3	9.65	92.8**)	1.6	9.84
Tyrosine	4.15	88.7	1.9	3.68	93.0*)	2.2	3.86
Phenylalanine	4.52	88.4	3.2	4.00	92.2	2.6	4.17
Histidine	2.63	92.4**)	0.6	2.43	93.5**)	2.5	2.46
Arginine	4.33	91.5	2.0	3.96	95.2***)	2.8	4.12
N in % of dry matter (maize)		1.61			1.61		
N in % of dry matter (diet)		1.49			1.50		
Protein value expressed as a percentage: TD		87.6	2.2		90.2	2.6	
BV		58.1	2.5		72.6	3.6	
NPU		50.9	2.9		65.5	3.8	
UN		0.82	0.05		1.06	0.06	

G. Experiments with sorghum

In work by *Vaverich et al.* (1959) with chicks, sorghum was shown to be too low in lysine. Lysine supplements improved the growth of chicks, but arginine supplements failed to result in growth improvements in these experiments. Lysine and methionine supplements to pigs (*Clausen* 1961b) improved both daily gain and slaughter quality. Furthermore low protein sorghum was found to be superior to the protein of the high protein sorghum as indicated by growth of chicks fed at constant level of protein in the diet (*Vaverich et al.* 1959). Similar results were obtained by *Waggle et al.* (1966) with rats. The high protein sorghum, however, had higher percentages of all of the 17 amino acids studied compared with the low protein sorghum. *Bragg et al.* (1969) found significant differences in the TD of individual amino acids in experiments with chicks. The value obtained for lysine was 87.9,

Table 23. AAC, AAA plus TD, BV, NPU and UN of sorghum determined in experiments with rats and baby pigs

Tabel 23. ASS, TAS samt SF, BV, NPU og UN i milokorn bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	1.83	72.7***)	2.9	1.33	71.5***)	3.3	1.31
Methionine	1.72	83.8	3.3	1.44	84.6	2.6	1.46
Cystine	1.25	84.6	2.8	1.06	78.5***)	3.3	0.98
Aspartic acid	7.50	82.5	2.9	6.19	83.0	2.8	6.23
Threonine	3.61	79.5	6.2	2.87	82.7	2.3	2.99
Serine	4.61	100.0***)	2.6	4.61	90.4***)	2.2	4.17
Glutamic acid	21.24	90.0*)	1.4	19.12	88.1**)	2.1	18.71
Glycine	3.00	82.6	3.9	2.48	77.7***)	3.8	2.33
Alanine	9.75	91.6**)	1.4	8.93	87.2*)	2.2	8.50
Valine	5.41	84.4	3.1	4.57	84.8	2.4	4.59
Isoleucine	4.50	81.9	3.6	3.69	83.3	3.9	3.75
Leucine	11.63	92.1**)	1.4	10.71	87.0	2.0	19.12
Tyrosine	4.06	88.1	3.8	3.58	86.5	2.5	3.51
Phenylalanine	5.17	88.7	4.7	4.59	85.4	1.9	4.42
Histidine	2.00	89.0	2.6	1.78	85.2	2.3	1.70
Arginine	3.39	88.6	3.5	3.00	87.2*)	2.5	2.96
N in % of dry matter (sorghum)		2.06			2.06		
N in % of dry matter (diet)		1.48			1.52		
Protein value expressed as a percentage: TD		84.8	3.1		85.3	2.6	
BV		52.2	2.7		73.5	2.5	
NPU		44.3	3.5		62.7	3.0	
UN		0.99	0.08		1.29	0.06	

while methionine and cystine were measured to 93.2 and 95.8 respectively, i. e., these values seem to be rather high. Results from present investigation with sorghum are shown in Table 23.

As shown in Table 23, the TD of total N is similar for both rats and pigs and approximates 85%. BV, however, is considerably higher, about 21 units, when measured on pigs compared to the BV value obtained with rats. In view of the low lysine content of sorghum, the BV value obtained with pigs is exceptional high, particularly when the extremely low TD of lysine is taken into account, available lysine being only 1.31 g/16 g N. However, there were considerable difficulties in inducing experimental animals to accept the sorghum diet and corrections for feed residue were necessary. Since coprophagy was also observed in these animals, the results obtained with pigs fed the sorghum diet should be treated with caution.

A close agreement can be seen between the TD values of the individual

amino acids obtained with rats and pigs. The TD of lysine is significantly lower than for total N, while glutamic acid, leucine, histidine and arginine have higher TD values. However, these values are all significantly lower than those reported by *Bragg et al.* (1969).

H. Experiments with casein

Casein is very rich in protein and also possesses a favourable amino acid composition. However, BV is surprisingly low when measured on rats and supplements of methionine and lactose have, as will be discussed, a very positive influence on N retention. The relatively low BV (60–70) of casein have been demonstrated by many workers (*Beadles et al.* 1933, *Kik* 1938, *Huges & Hauge* 1945, *Block & Mitchell* 1946–47). In spite of the similar amino acid contents of casein and skim milk powder, the BV of skim milk powder is found to be much higher (80–90%) than that of casein (*Fairbanks & Mitchell* 1935, *Summer* 1938, *Block & Mitchell* 1946–47, *Richter & Schiller* 1956). The favourable response of a methionine supplement to casein has been verified by *Kinsey & Grant* 1944, *Albanese & Frankstone* 1945, *Miller & Bender* 1955, *Baron* 1958 and *Schiller & Ocio* 1963. The addition of methionine to a casein diet, however, has produced little response in pigs, although considerable reaction has been found with rats (*Schiller & Ocio* 1963). These experiments indicate a lower requirement of sulphur-bearing amino acids in pigs compared to rats. Average TD and BV values of 98.0 and 85.6 respectively were measured for pigs. In the experiments with rats TD values between 96 and 99 and BV values from 61 to 71 were obtained. It is of interest to note that, although the TD values obtained in the two animal species show close agreement, the BV is much lower in rats than in pigs despite a similar protein content in the two diets (approximately 10%).

Poppe et al. (1969a) reported TD values of 98.2, 99.9 and 99.5 for total N, lysine and methionine respectively in experiments with rats. These figures are much in line with results obtained by *Carlson & Bayley* (1970) in experiments with baby pigs in which TD values for total N and for amino acids were within the range 97–100%. By sampling prior to the terminal ileum of chicks instead of taking faeces, *Soares & Kifer* (1971) measured a TD of 97 for both lysine and methionine.

This discussion indicates that the total N and the individual amino acids in casein are readily absorbed. The results of the present investigation support this conclusion.

From the results given in Table 24 it can be seen that total N in casein is completely digested with a TD of 101.1 when measured on rats and 99.4% when measured on pigs. BV, however, was 71.9 in the experiment with rats but 84.4 in pigs. This situation is very much the same as that reported by *Schiller & Ocio* (1963).

Table 24. AAC, AAA plus TD, BV, NPU and UN of casein determined in experiments with rats and baby pigs

Tabel 24. ASS, TAS samt SF, BV, NPU og UN i kasein bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	8.20	100.1	1.2	8.21	99.7	0.7	8.18
Methionine	3.22	100.6	0.6	3.24	100.3	1.0	3.23
Cystine	0.48	97.2	4.1	0.47	100.1	5.8	0.48
Aspartic acid	8.39	100.6	3.0	8.44	98.9	1.3	8.30
Threonine	4.31	100.6	2.8	4.34	98.8	1.5	4.26
Serine	6.66	100.8	3.2	6.71	100.9**)	0.9	6.72
Glutamic acid	30.04	97.1*)	2.2	29.17	99.6	0.5	29.92
Glycine	1.85	99.9	4.6	1.85	97.7	3.2	1.81
Alanine	3.14	100.4	2.9	3.15	99.9	2.5	3.14
Valine	6.59	99.1	2.5	6.53	99.1	1.0	6.53
Isoleucine	6.24	96.1*)	2.8	5.99	99.1	0.9	6.18
Leucine	11.97	101.5	1.6	12.15	99.3	0.7	11.89
Tyrosine	3.40	102.0	1.4	3.47	98.7	1.3	3.36
Phenylalanine	5.24	100.9	2.2	5.29	98.8	1.4	5.18
Histidine	3.38	101.0	0.3	3.41	99.9	0.7	3.38
Arginine	3.56	100.9	1.5	3.59	99.2	1.5	3.53
N in % of dry matter (casein)		15.02			15.02		
N in % of dry matter (diet)		1.49			3.04		
Protein value expressed as a percentage: TD		101.1	0.9		99.4	1.2	
BV		71.9	2.8		84.4	4.9	
NPU		72.7	3.4		83.9	5.0	
UN		10.92	0.51		12.60	0.75	

Since the protein in casein is completely digested the TD of the amino acids must in general approximate one hundred. The results in Table 24 demonstrate that this is the case for almost all of the amino acids in both experiments with rats and pigs. Due to these high TD values, available amino acids (AAA) will be almost identical with amino acid composition (AAC).

I. Experiments with fish meal

Fish is known to be a good source of protein of excellent quality. *Laksesvela* (1961) has given a very broad discussion of herring meal and various herring products. This work demonstrates the superior quality of fish protein.

In experiments with rats, *Nehring & Bock* (1961) recorded TD values of 90.4 to 95.1 for fish meals of different origin; BV varied from 69.3 to 83.6. Similar results were obtained by *Eggum* (1968b) in experiments with rats

fed different types of fish protein. *Wünsche & Bock* (1965) found the TD and BV of fish meal of excellent quality in rats to be 93.0 and 80.9 respectively, while *Njaa et al.* (1966) found that the estimated net protein utilization of herring meal was much affected by the method of processing.

Hennig (1957) determined the TD and BV of fish meal in pigs and obtained values of 96.4 and 76.0 respectively. *Homb* (1962) found corresponding values of 90 and 88 in experiments with herring meal and *Schiller & Schulz* (1970) recorded almost identical values for herring meal fed to rats.

In experiments with Peruvian fish meal fed to adult pigs, *Dammers* (1964) obtained TD values for individual amino acids of approximately 90 or slightly above. *Poppe et al.* (1969a) measured TD values of 93.6, 95.4 and 94.9 for total N, lysine and methionine in fish meal from Rostock. *Waring* (1969), employing colostomized hens, obtained TD values of 93.5, 91.7 and 83.0 for lysine, methionine and cystine respectively. Figures published by *FAO*

Table 25. AAC, AAA plus TD, BV, NPU and UN of fish meal determined in experiments with rats and baby pigs

Tabel 25. ASS, TAS samt SF, BV, NPU og UN i fiskemel bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	7.90	96.8*)	1.3	7.65	95.6*)	2.4	7.55
Methionine	2.78	94.9	1.9	2.64	93.7	3.5	2.60
Cystine	1.01	93.9	1.7	0.95	93.5	2.4	0.94
Aspartic acid	10.84	94.4	1.9	10.23	94.9	2.9	10.29
Threonine	5.00	95.1	1.3	4.76	95.3	2.9	4.77
Serine	4.73	100.0***)	1.0	4.73	97.0**)	2.9	4.59
Glutamic acid	14.94	96.0*)	1.3	14.34	95.7*)	2.6	14.30
Glycine	6.78	95.4	1.0	6.47	94.1	2.6	6.38
Alanine	6.95	95.3	2.4	6.62	94.6	3.1	6.57
Valine	5.75	94.7	1.5	5.45	94.1	3.4	5.41
Isoleucine	4.78	93.7	1.9	4.48	93.6	3.3	4.47
Leucine	7.94	96.0*)	1.1	7.62	94.6	3.2	7.51
Tyrosine	3.14	92.1	2.5	2.89	93.3	3.6	2.93
Phenylalanine	5.18	92.3	2.1	4.78	88.5*)	6.2	4.58
Histidine	2.22	97.6**)	0.8	2.17	95.5	3.1	2.12
Arginine	5.78	97.2**)	0.7	5.62	96.5**)	2.3	5.58
N in % of dry matter (fish meal)		12.63			12.63		
N in % of dry matter (diet)		1.52			2.98		
Protein value expressed as a percentage: TD		93.8	0.8		93.2	3.2	
BV		76.3	2.9		90.1	2.7	
NPU		71.6	2.9		84.0	4.8	
UN		9.04	0.37		10.61	0.61	

(1970) show available lysine and methionine to be 93.5 and 91.4 respectively in white fish meal when determined in colostomized hens.

Fish meal would thus in general appear to be an excellent protein source with high TD values for the component amino acids. Table 25 shows the results of the present experiments.

The sample of fish meal (Icelandic) employed in these investigations was found to have a TD close to 93% in experiments with both rats and pigs. Again BV is considerably higher when measured with pigs (90.1) than with rats (76.3). However, these results agree closely with data obtained by other workers.

The TD of the individual amino acids can be seen to differ very little from the TD of total N, although the basic amino acids lysine, histidine and arginine all have significantly higher values. Furthermore the TD values obtained with rats are very similar to those from pigs.

J. Experiments with meat and bone scraps

Several investigations have demonstrated that an increasing concentration of meat and bone scraps in diets for monogastric animals has a negative effect on daily gain and performance (*Peo & Hudman* 1962, *Hansen* 1963, *Baelum & Petersen* 1966). *Gartner & Burton* (1965) found that both daily gain and feed utilization decreased with increasing ash content in meat and bone scraps. This is in agreement with experiments carried out by *Eggum* (1970b) who showed that the nitrogen content in meat and bone scraps influences both amino acid composition and the biological criteria TD, BV and NPU. Lysine and methionine + cystine are positively correlated with nitrogen content, whereas the correlation is negative in the case of glycine. Thus TD, BV and NPU are all positively correlated with N content. *Nehring & Bock* (1961) obtained a TD of 78.5 and BV of 61.3 in experiments with rats fed a sample rich in protein, whereas in a sample with a lower protein content (*Wünsche & Bock* 1965) TD was found to be 76.9 while BV was only 51.4. In a comprehensive study of meat and bone scraps manufactured by different processes, *Richter et al.* (1962) showed that TD varied from 82.3 to 87.7 while BV varied from 25.8 to 40.8.

The TD of the individual amino acids in meat and bone scraps was found by *Waring* (1969), using colostomized fowl, to lie within the range of ± 5% of the mean. The TD for lysine, methionine and cystine was 73.4, 73.6 and 58.7 respectively, while the digestibility of aspartic acid was only 52.4%. *Atkinson & Carpenter* (1970) concluded from chick growth experiments that the lysine, methionine and tryptophan of meat meals are approximately 80% available and that dilution of muscle protein with tendon and ossein in the raw material is at least as important as processing damage in reducing the

quality of the products. TD for commercial meat meal samples was found to be 85% in experiments with chicks (*Atkinson & Carpenter* 1970). In experiments with rats NPU values of approximately 32 were obtained. *Dammers* (1964) showed that TD of the individual amino acids in meat and bone scraps approximated the TD of total N (88.0) in adult pigs. The one exception was cystine; this amino acid was absorbed with only 65.4%.

It can be seen from this discussion that marked differences exist between the various estimates of protein quality in meat and bone scraps. This is not surprising since the manufacturing process can differ from one factory to another, just as differences exist in the raw materials employed. In general, however, the protein quality in meat and bone scraps is relatively low, a conclusion also supported by the present experiments (Table 26).

Table 26. AAC, AAA plus TD, BV, NPU and UN of meat and bone scraps determined in experiments with rats and baby pigs

Tabel 26. ASS, TAS samt SF, BV, NPU og UN i kødbenmel bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	5.40	87.3	1.7	4.71	85.7	2.4	4.63
Methionine	1.34	74.7***)	2.3	1.00	80.1***)	3.0	1.07
Cystine	0.82	69.5*)	10.1	0.57	72.4***)	5.7	0.59
Aspartic acid	7.59	82.5***)	1.1	6.25	84.5	3.5	6.41
Threonine	3.28	82.4**)	1.7	2.70	82.6*)	3.2	2.71
Serine	3.80	85.3**)	0.4	3.24	87.6	3.1	3.33
Glutamic acid	12.78	78.4***)	0.8	10.02	86.4	2.3	11.04
Glycine	15.82	94.4***)	0.7	14.93	92.8***)	2.4	14.68
Alanine	7.20	92.0**)	0.9	6.62	86.3	2.8	6.21
Valine	4.14	82.3***)	1.1	3.41	80.4***)	2.8	3.33
Isoleucine	2.92	70.9***)	1.2	2.07	79.3***)	2.7	2.32
Leucine	6.18	85.6*)	1.0	5.29	82.9*)	2.3	5.12
Tyrosine	2.48	80.0***)	1.3	1.98	80.9**)	4.2	2.01
Phenylalanine	3.44	85.6	5.8	2.94	82.0**)	2.5	2.82
Histidine	1.97	92.6**)	1.3	1.82	86.5	4.6	1.70
Arginine	6.43	93.7***)	1.0	6.02	90.7***)	1.9	5.83
N in % of dry matter (meat and bone scraps)		8.82			8.82		
N in % of dry matter (diet)		1.52			3.03		
Protein value expressed as a percentage: TD		87.5	3.4		85.5	2.6	
BV		48.2	5.7		64.6	2.7	
NPU		42.3	6.1		55.2	2.3	
UN		3.73	0.54		4.87	0.20	

As shown in Table 26, the contents of methionine and cystine are extremely low, although the lysine content is reasonably satisfactory. The concentration of glycine is very high, indicating the presence of considerable amounts of collagen in the material. TD of total N is 87.5 when measured on rats and 85.5 in the case of pigs, i.e., very much the same. As in several of the other diets discussed, BV is much higher (64.6) when measured on pigs than when rats are employed (48.2). The TD of the individual amino acids varies to a certain extent and the low values for the sulphur-containing amino acids should be emphasized. The standard deviation for TD of cystine is relatively high, although the values are similar to those discussed above. As the carbohydrate concentration in the raw material is low, the absorbability of lysine does not appear to be influenced by processing (see later). In general the results in Table 26 indicate a relatively poor protein quality in meat and bone scraps.

K. Experiments with soya bean meal

Soya bean protein is rich in lysine but poor in the sulphur-containing amino acids. This specific aminogram renders soya bean a suitable protein supplement to grain protein. In experiments with rats (*Eggum* 1967a) it was found possible to increase BV from 62.0 to 85.6 by means of a methionine supplement. The simultaneous addition of threonine gave a further increase in BV to 94.8. These results demonstrate the value of BV in determining the limiting amino acid. *Schiller* (1968) also reported a large positive effect when methionine was added to soya bean meal; BV was increased from 68.6 to 83.7 and a TD of 94.7 was obtained. *Wünsche & Bock* (1965) recorded a somewhat lower TD (87.8) but the same BV (68.6) as found by Schiller. In experiments with pigs *Hennig* (1957) obtained a TD of 97.3 and a BV of 54.8 while *Nehring & Bock* (1961) reported a BV of 60 in pigs but 65 when measured in rats.

Methionine balance studies described by *Melnick et al.* (1946) showed that 49% of the methionine in a sample of soya bean meal appeared in the faeces and hence was not available to the rat. This value is much lower than that obtained by *Poppe et al.* (1969a) with rats; in these experiments 85.3% of the methionine in soya bean meal was absorbed while lysine and total N had TD values of 90.5 and 87.5 respectively. These values for lysine and methionine are within the same range as the results obtained with rats (*FAO* 1970) in which 87.2% and 91.1% of the lysine and methionine respectively was absorbed. For colostomized hens (*FAO* 1970) the corresponding figures were 90.9 and 87.6 respectively. By analysing the ileal contents of chicks, *Soares & Kifer* (1971) measured an absorption of 82% for lysine and 80% for methionine. An average absorbability of 79% was obtained for all amino

acids, a value which appears to be rather low. When calculated on the basis of weight gain in chicks, *Guo et al.* (1970) obtained a TD of lysine in soya bean meal of 96.6. *Dammers* (1964) and *Meier et al.* (1970) found in experiments with pigs that the absorbability for all amino acids in soya bean meal was extremely high – above 90 and with certain values close to 100%. A TD of 95.3 was obtained for total N (*Dammers* 1964). However, in experiments with baby pigs *Carlson & Bayley* (1970) obtained significant differences in the absorbability of the individual amino acids, alanine having the lowest value of 82%, while 93% of the glutamic acid was absorbed. The present investigation also showed high TD values in the protein of soya bean meal. The TD of total N can be seen from the results given in Table 27 to be the same for both rats and pigs, i.e., approximately 90%. Again BV is lower when measured in rats (62.0) than when measured in pigs (71.2). These values

Table 27. AAC, AAA plus TD, BV, NPU and UN of soya bean meal determined in experiments with rats and baby pigs

Tabel 27. ASS, TAS samt SF, BV, NPU og UN i sojaskrå bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	5.98	91.6	2.2	5.48	91.7	2.2	5.48
Methionine	1.61	88.9	2.9	1.43	87.4*)	3.0	1.41
Cystine	1.56	92.4	1.5	1.44	91.3	2.4	1.42
Aspartic acid	10.68	92.8	1.6	9.91	93.2**)	1.9	9.95
Threonine	3.73	88.4	2.6	3.30	89.6	2.3	3.34
Serine	4.95	100.0***)	1.6	4.95	94.3***)	1.8	4.67
Glutamic acid	17.82	95.6**)	1.6	17.04	95.1***)	1.5	16.95
Glycine	4.15	86.6*)	2.1	3.59	88.8	2.3	3.69
Alanine	4.19	87.2	2.3	3.65	85.8**)	3.6	3.60
Valine	5.02	89.5	2.1	4.49	89.4	2.5	4.49
Isoleucine	4.53	89.2	2.1	4.04	89.7	2.6	4.06
Leucine	7.48	90.3	1.7	6.75	90.8	2.4	6.79
Tyrosine	3.03	88.2	4.3	2.67	90.4	3.1	2.74
Phenylalanine	5.21	89.1	2.5	4.64	90.8	2.2	4.73
Histidine	3.36	96.1**)	1.3	3.23	94.5***)	2.1	3.18
Arginine	7.15	96.7**)	1.5	6.91	96.2	1.3	6.88
N in % of dry matter (soya bean meal)		8.20			8.20		
N in % of dry matter (diet)		1.48			2.97		
Protein value expressed as a percentage: TD		90.7	1.6		90.2	2.6	
BV		62.0	3.3		71.2	3.8	
NPU		56.2	3.6		64.2	4.2	
UN		4.61	0.29		5.27	0.35	

are much the same as those found in the literature. The TD of the individual amino acids is very similar when measured in the two animal species and approximately 90% in most cases. However, as was also found in cereals, glutamic acid, histidine and arginine all have higher absorbabilities than total N, while alanine and glycine have lower TD values. The present TD values of amino acids are in general somewhat lower than the values of *Dammers* (1964), but more in line with results obtained by *Poppe et al.* (1969a), *FAO* (1970) and *Carlson & Bayley* (1970).

L. Experiments with groundnut meal

Groundnut protein shows a poor aminogram. The amino acid deficiency is particularly pronounced in the case of the most important acids such as lysine, methionine and threonine (*Clausen* 1961c). *Bunyan & Price* (1960) obtained with rats TD and BV values of 80 and 52 respectively for 6 groundnut samples. *Wünsche & Bock* (1965) reported a TD of 91.1 and a BV of 47.5 in extracted groundnut meal, whereas the pressed material had a TD of 92.4 and a BV of 56.1 when measured on rats. *Schiller* (1968) obtained TD and BV values of 92.2 and 49.8 respectively in rats, i.e., much the same as *Wünsche & Bock* (1965). *Schiller* (1968) also supplemented groundnut meal with lysine and methionine and obtained an increase in BV from 49.8 to 62.3.

The absorbability of the individual amino acids determined in rats was reported by *Kuiken* (1952) to be very high in groundnut meal, ranging from 94.8 to 99.5. *Poppe et al.* (1969a), however, obtained the somewhat lower values of 90.7, 88.9 and 83.5 for total N, lysine and methionine respectively. *Dammers* (1964) found TD values in adult pigs of approximately 90% for all amino acids with the exception of cystine which had a TD of 78.5. The values obtained by *Poppe et al.* (1969a) and *Dammers* (1964) are, however, very similar to those obtained in present work (Table 28).

The TD values for total N are almost the same for both rats (92.2) and pigs (91.2) and agree well with the values obtained by other workers (*Meier et al.* 1970a, *Meier et al.* 1970b). The fact that similar BV values are found in both rats and pigs is rather surprising, particularly in view of the higher BV values generally obtained with pigs. The BV values obtained for rats in the present experiments are, however, much higher than the values reported by *Wünsche & Bock* (1965) and *Schiller* (1968). Furthermore the content of both sulphur-containing amino acids and lysine is very low in groundnut meal. Such a situation might well cause a relatively low BV when measured in pigs fed a diet with a relatively high N content.

The TD of several of the individual amino acids differs significantly from the TD of total N and the agreement between values obtained in pigs and in rats is not as good as in most of the other samples studied.

Table 28. AAC, AAA plus TD, BV, NPU and UN og groundnut meal determined in experiments with rats and baby pigs

Tabel 28. ASS, TAS samt SF, BV, NPU og UN i jordnødskrå bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	3.19	86.9**)	2.1	2.77	88.1**)	3.2	2.81
Methionine	1.13	81.0**)	4.9	0.92	88.7*)	2.7	1.00
Cystine	1.11	95.0	4.5	1.05	89.1**)	1.7	0.99
Aspartic acid	11.75	94.4	1.7	11.09	93.1**)	1.3	10.94
Threonine	2.74	81.6***)	3.6	2.24	86.9***)	1.9	2.38
Serine	4.60	100.4***)	2.3	4.62	94.1***)	1.5	4.33
Glutamic acid	19.94	91.3	2.0	18.21	95.1	0.8	18.96
Glycine	5.71	89.9*)	1.2	5.13	87.8**)	3.1	5.01
Alanine	3.70	88.3*)	2.8	3.27	88.8**)	1.9	3.29
Valine	3.92	84.6***)	2.4	3.32	89.6	2.2	3.51
Isoleucine	3.49	77.6***)	2.5	2.71	89.6*)	2.0	3.13
Leucine	6.17	92.1	1.9	5.68	91.1	2.0	5.62
Tyrosine	4.19	88.8	9.2	3.72	92.6	1.9	3.88
Phenylalanine	4.90	96.9	6.8	4.75	91.6	1.9	4.49
Histidine	2.11	100.2***)	2.1	2.11	92.1	1.9	1.94
Arginine	8.42	101.1***)	0.4	8.51	95.4***)	0.8	8.03
N in % of dry matter (groundnut meal)		8.51			8.51		
N in % of dry matter (diet)		1.50			3.02		
Protein value expressed as a percentage: TD		92.2	3.3		91.2	1.6	
BV		60.4	4.1		59.7	4.4	
NPU		55.8	5.7		54.4	4.6	
UN		4.75	0.49		4.63	0.39	

M. Experiments with sunflower seed meal

Sunflower seed meal is known to be a good source of sulphur-bearing amino acids and threonine but a relatively poor source of lysine (*Clausen* 1961d). *Schiller* (1968) demonstrated that lysine is the primary limiting amino acid in sunflower seed meal. She found a BV of 60.5 in unsupplemented diets to rats, while a lysine supplement increased BV to 76.2. A TD value of 87.2 was obtained. *Wünsche & Bock* (1965) reported a TD of 94.6 and a BV of 71.0. Very few data are available on the TD of the individual amino acids; *Poppe et al.* (1969a) obtained TD values in rats of 88.3, 90.4 and 86.2 for lysine, methionine and total N. Results from the investigation of sunflower seed meal are shown in Table 29.

Table 29. AAC, AAA plus TD, BV, NPU and UN of sunflower seed meal determined in experiments with rats and baby pigs

Tabel 29. ASS, TAS samt SF, BV, NPU og UN i solsikkeskrå bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	3.50	88.0	4.9	3.08	86.9**)	3.0	3.04
Methionine	2.21	93.6	3.0	2.07	92.3*)	2.1	2.04
Cystine	1.49	93.6	1.4	1.39	90.9	1.2	1.36
Aspartic acid	10.34	93.1	2.8	9.63	99.2**)	1.1	10.26
Threonine	4.05	89.4	2.5	3.62	89.9	1.8	3.64
Serine	4.53	100.1***)	1.7	4.53	94.2***)	1.4	4.27
Glutamic acid	23.98	98.5**)	1.2	23.62	96.0	0.8	23.02
Glycine	5.88	91.3	1.5	5.37	91.5	1.7	5.38
Alanine	4.42	88.2	3.6	3.90	88.6	2.9	3.92
Valine	5.10	92.6	1.9	4.72	91.3	1.9	4.66
Isoleucine	4.70	92.7	2.0	4.36	91.4	2.3	4.30
Leucine	6.56	91.8	2.1	6.02	91.6	1.5	6.01
Tyrosine	3.06	92.4	1.5	2.83	89.2	3.0	2.73
Phenylalanine	4.86	94.6	3.1	4.60	90.6	1.9	4.40
Histidine	2.86	99.3**)	1.7	2.84	95.1***)	1.1	2.72
Arginine	8.02	100.2***)	1.3	8.04	96.3***)	0.7	7.72
N in % of dry matter (sunflower seed meal)		6.55			6.55		
N in % of dry matter (diet)		1.52			2.96		
Protein value expressed as a percentage: TD		91.9	1.0		90.4	1.7	
BV		70.7	2.7		60.4	3.6	
NPU		64.9	2.7		54.6	3.9	
UN		4.25	0.18		3.58	0.25	

Close agreement is obtained between the TD values for total N as determined in rats (91.9) and pigs (90.4). BV, however, is considerably higher in experiments with rats than with pigs, 70.7 and 60.4 respectively. The BV obtained with rats is within the range which might be expected in view of the relatively high content of sulphur-bearing amino acids. The relatively low BV obtained with pigs is probably due to the low content of lysine. The level of available lysine is very much the same in both sunflower seed meal and groundnut meal and since lysine is the primary limiting amino acid similar BV values of approximately 60 would be expected. The aminogram of sunflower seed meal is very similar to that of barley, although the BV of sunflower seed meal is considerably lower than in barley as determined in pigs. This latter difference is no doubt due to the different N contents in the experimental diets. Sunflower seed meal was fed at the 3.0% N level in the diet whereas barley was fed with only 1.5% N.

The TD of the individual amino acids is very similar in rats and pigs and similar to the TD of total N with a value of approximately 90%. This is in agreement with the values for lysine and methionine reported by *Poppe et al.* (1969a). As for most of the samples evaluated, the TD values for glutamic acid, histidine and arginine are significantly higher than for total N.

N. Experiments with skim milk powder + dehulled oats

A combination of milk and oat protein will provide a favourable amino acid composition and should result in high BV values. The data from these experiments are shown in Table 30.

The TD of total N in this combination is approximately 90, intermadiate between the values for skim milk powder (above 90, *Eggum et al.* 1970) and oats (below 90, Table 19). Again a higher BV is found in rats (77.6) than in pigs (71.1). In this connection it should be emphasized that the nitrogen

Table 30. AAC, AAA plus TD, BV and NPU of skim milk powder + dehulled oats determined in experiments with rats and baby pigs

Tabel 30. ASS, TAS samt SF, BV og NPU i skummetmælkspulver + afskallet havre bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	5.11	89.2	2.3	4.56	91.1	3.6	4.66
Methionine	2.10	90.1	1.9	1.89	90.5	3.8	1.90
Cystine	1.88	96.1	1.8	1.72	91.9*)	2.6	1.73
Aspartic acid	7.34	88.3	2.4	6.48	88.5	2.9	6.50
Threonine	3.66	89.6	2.6	3.28	90.3	3.0	3.30
Serine	4.89	95.3***)	1.0	4.66	94.6***)	2.7	4.63
Glutamic acid	21.62	94.7***)	2.3	20.47	94.1***)	1.4	20.34
Glycine	3.22	86.8	2.0	2.79	84.8**)	3.7	2.73
Alanine	3.78	84.3**)	3.1	3.19	82.9**)	5.6	3.13
Valine	5.87	89.0	1.9	5.22	90.2	2.7	5.29
Isoleucine	4.46	88.2	3.0	3.93	90.4	2.8	4.03
Leucine	8.26	93.6***)	1.4	7.73	93.1***)	2.0	7.69
Tyrosine	4.07	90.4	1.7	3.68	92.4**)	2.4	3.76
Phenylalanine	4.68	88.6	1.8	4.15	90.5	2.9	4.24
Histidine	2.23	93.1**)	2.2	2.08	92.5**)	2.5	2.06
Arginine	4.35	93.4***)	1.9	4.06	92.1*)	2.6	4.01
N in % of dry matter (diet)		1.52			2.02		
Protein value expressed as a percentage: TD		88.3	2.3		89.3	2.4	
BV		77.6	2.8		71.1	1.8	
NPU		68.5	3.0		63.5	2.5	

content in the pig diets was 2.0%, compared with 1.5% in the diets fed to rats. However, a possible explanation of this difference in BV values is that whereas cystine or methionine is probably the limiting amino acid in rats fed skim milk powder and dehulled oats, lysine is probably the limiting factor in the case of pigs. Nevertheless a higher BV for pigs might have been expected. It should, however, be noted that the pigs used in this trial were Danish Landrace x Gloucester Old Spot which might have had a lower ability to utilize absorbed nitrogen than pigs of pure Danish Landrace.

The TD of the individual amino acids is in most cases similar to the TD of total N, although glycine and alanine both have significantly lower values while glutamic acid, histidine and arginine show higher values.

O. Experiments with soya bean meal + dehulled oats

A combination of lysine-rich soya bean protein and oat protein rich in sulphur-containing amino acids should theoretically give high BV values. The

Table 31. AAC, AAA plus TD, BV and NPU of soya bean meal + dehulled oats determined in experiments with rats and baby pigs

Tabel 31. ASS, TAS samt SF, BV og NPU i sojaskrå + afskallet havre bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	4.46	84.3	2.1	3.76	84.2	3.3	3.76
Methionine	1.73	81.0**)	1.7	1.40	81.2**)	4.0	1.40
Cystine	2.22	90.9**)	2.0	2.02	89.9**)	2.3	2.00
Aspartic acid	8.97	86.2	2.4	7.73	86.3	3.1	7.74
Threonine	3.31	84.0	1.9	2.78	82.5*)	3.6	2.73
Serine	4.60	90.9*)	3.0	4.18	90.2**)	2.6	4.15
Glutamic acid	19.92	92.4**)	1.8	18.41	91.3***)	1.8	18.19
Glycine	4.34	83.8	2.1	3.64	82.4**)	3.0	3.58
Alanine	4.29	77.6***)	2.1	3.33	75.7***)	4.8	3.25
Valine	5.16	84.8	2.0	4.38	82.3*)	4.8	4.25
Isoleucine	4.13	85.2	1.9	3.52	84.3	2.9	3.48
Leucine	7.25	85.8	1.8	6.22	86.9	2.3	6.30
Tyrosine	2.96	84.9	2.6	2.51	86.4	3.3	2.56
Phenylalanine	4.78	86.0	1.7	4.11	86.1	3.0	4.12
Histidine	2.49	91.0**)	1.8	2.27	91.6***)	2.4	2.28
Arginine	6.31	91.6**)	2.1	5.78	92.3***)	1.9	5.82
N in % of dry matter (diet)		1.51			1.98		
Protein value expressed as a percentage: TD		85.9	2.0		86.1	3.0	
BV		77.4	2.4		75.1	2.3	
NPU		66.5	2.6		64.7	3.6	

amino acid composition of this mixture and the experimental results are shown in Table 31.

The TD of this mixture is the same when determined on rats (85.9) and pigs (86.1) and is intermediate to the values obtained when soya bean meal (Table 27) and oats (Table 19) are fed separately. The BV obtained with rats (77.4) is exactly the same as the value obtained with the combination of skim milk powder + dehulled oats (Table 30). This is probably due to the fact that the sulphur-containing amino acids are limiting in this diet – and at the same level as in the previous mixture. However, the higher BV value in pigs of this diet compared to the former is difficult to understand since the content of essential amino acids is lower in the present diet. This might possibly be due to the fact that pigs of pure Danish Landrace were used in this trial, whereas crossbred pigs were used in the trial with skim milk powder + dehulled oats.

The trend in the TD values of the individual amino acids is very similar

Table 32. AAC, AAA plus TD, BV and NPU of »rød laktal« determined in experiments with rats and baby pigs

Tabel 32. ASS, TAS samt SF, BV og NPU i rød laktal bestemt i forsøg med rotter og grise

	AAC (g/16gN)	TD (rats) (%)	(s)	AAA (g/16gN)	TD (pigs) (%)	(s)	AAA (g/16gN)
Lysine	5.90	89.6	1.8	5.29	93.1	1.9	5.49
Methionine	1.94	94.1***)	1.4	1.83	95.9***)	0.8	1.86
Cystine	1.45	88.7	2.1	1.29	92.4	1.2	1.34
Aspartic acid	7.63	90.3	0.9	6.89	92.3	1.9	7.04
Threonine	4.07	92.1	1.5	3.75	94.0*)	1.6	3.83
Serine	5.14	93.9**)	1.7	4.83	95.7***)	1.2	4.92
Glutamic acid	20.15	95.1***)	1.7	19.16	96.0***)	1.2	19.34
Glycine	2.98	88.1	2.2	2.63	87.6***)	2.1	2.61
Alanine	3.84	87.3	2.0	3.35	87.9***)	2.4	3.38
Valine	5.91	94.2**)	1.6	5.57	96.5***)	0.9	5.70
Isoleucine	4.61	91.2	1.4	4.20	93.6	1.7	4.31
Leucine	9.98	93.0*)	2.3	9.28	95.6***)	1.3	9.54
Tyrosine	4.57	93.0*)	2.4	4.25	95.3***)	1.3	4.36
Phenylalanine	4.88	91.2	1.9	4.45	93.8*)	2.0	4.58
Histidine	2.31	95.9***)	1.1	2.22	98.2***)	0.7	2.27
Arginine	4.46	87.8	1.6	3.92	90.9*)	1.8	4.05
N in % of dry matter (diet)		1.49			3.84		
Protein value expressed as a percentage: TD		89.9	1.4		92.4	1.3	
BV		85.2	2.1		78.1	2.2	
NPU		76.7	2.4		72.2	2.0	

in both rats and pigs; low values are found for methionine, glycine and alanine, while glutamic acid, histidine and arginine have significantly higher TD values than total N. This situation is much the same as that described for the previous diet (Table 30).

P. Experiments with prestarter for baby pigs (Rød laktal)

»Rød laktal« is made up of several protein-carrying components in order to render the mixture suitable for baby pigs. It appears from Table 32 that this combination gives relatively high BV values.

The TD of the mixture is approximately 90%, 89.9% in the case of rats and 92.4 in pigs. The BV is extremely high in experiments with rats (85.2), indicating an excellent amino acid composition even for this animal species. However, the BV obtained with pigs (78.1) is also high, particularly when considering the high N content (3.84%) in the experimental diet.

The TD values of the individual amino acids are in most cases similar to the TD of total N, although certain amino acids show significantly higher values. Furthermore the tendency shown by those values is very similar in both rats and pigs.

Q. General discussion

1. Experimental procedure

The experimental technique employed was the same for all diets for rats and pigs respectively and thus only a brief description will be given. It should, however, be emphasized again that the N concentration in the pig diets was adjusted to different N levels. Only the cereal diets were adjusted to the same N content as in the rat diets (1.5% of dry matter); the concentrated feedstuffs were adjusted to a N content of 3.0%, the combination of dehulled oats with skim milk powder and with soya bean meal to 2.0% N and the milk substitute to 3.84% N in dry matter.

The experiments with rats were generally completed without problems of any kind, although feed residues could not be avoided in all cases. This was especially the case with sorghum and meat and bone scraps, where feed residues made up approximately 12% of the feed offered. Feed residues with wheat and groundnut meal amounted to 5–6% and in the other diets residues were observed only sporadically.

In the experiments with pigs feed residues occurred in the same diets as for rats, although the residues were generally larger on a percentage basis. Thus in sorghum and meat and bone scraps approximately 17% of the diet was not consumed, while feed residues in diets of wheat, rye, groundnut meal and casein amounted to 5–8%. As previously mentioned, coprophagy was

observed in the experiment with sorghum, rendering the results with this cereal somewhat unreliable. Although corrections for feed residues were made in all cases, a correct result for feed residues in experiments with pigs is for obvious reasons difficult to obtain. Consequently the standard deviations will be affected when feed residues are appreciable, such as in the diets with sorghum and meat and bone scraps.

In general the state of health of the pigs was good. Only in the experiments with casein and meat and bone scraps did the animals catch a cold, probably explaining in part the reduced appetite of animals fed these diets.

With the exception of feed residues in certain of the diets, all experiments were conducted according to plan.

2. True digestibility

The TD of total N in barley, oats and rye can be seen to be approximately 80, i.e., about 20% of the N content passes unabsorbed through the animals. This relatively large percentage of the N content is of no value for animal production and thus warrants further consideration. The situation is somewhat better in the other cereal proteins; approximately 85% of total N in sorghum is absorbed and almost 90% in wheat and maize.

The protein in casein is completely available to the organism, while in fish meal approximately 93% is absorbed. As previously discussed, the quality of meat and bone scraps depends on both the original material and the method of manufacture. In the present sample, however, TD was found to be approximately 87%. Nitrogen in soya bean meal, groundnut meal and sunflower seed meal shows an absorption of approximately 90% or above. The TD of the mixtures studied was found to be directly dependent on the TD of the individual N-carrying components involved.

From this discussion it can be seen that of the protein sources evaluated only casein protein is completely absorbed. The TD of the other feeds ranged from 80 to 93%, i.e., 7 to 20% of the potential nitrogen is unavailable for the animals in question.

The TD values of the individual amino acids are in most cases found to be approximately the same as the TD of total N in the diet consumed.

In cereal proteins (except maize), however, lower TD values were generally obtained for lysine, aspartic acid, glycine and alanine compared to total N, whereas higher values were found for glutamic acid, histidine and arginine. The low values for lysine are particularly unfortunate since this amino acid is the limiting factor in cereal proteins.

As discussed under barley, a possible explanation of these low lysine values is that the protein fraction having the lowest lysine content (prolamin) shows the highest digestibility of total N. It is, however, tempting to suggest that microbial protein synthesis in the intestine, rich in lysine, might give rise

to a higher lysine concentration in the faeces and hence to low TD values. However, a significant effect due to the microflora does not appear to be likely. Furthermore *Change & Hegsted* (1972) concluded that only a modest weight loss caused by a lysine-free diet is not due to the availability of lysine from faecal material.

The TD of the individual amino acids in concentrated feedstuffs does not generally differ from total N to the same extent as in cereal protein. Contrary to cereal protein low TD values for lysine are not found in concentrated feedstuffs, although a slight tendency in this direction was recorded in groundnut and sunflower seed meals. With the exception of lysine, the TD values in concentrated feeds are similar to the corresponding values in cereal proteins. In mixtures (samples 13, 14, 15) lysine appears to be absorbed to the same extent as the other amino acids, an observation also reported by *Just Nielsen* (1968, 1971).

The standard deviation of the TD values is relatively high in several cases, particularly in samples of low TD such as barley, oats, rye and to a certain extent groundnut meal. It is difficult to explain these high standard deviations but individual variation might well be more pronounced under conditions of greater nutritional stress.

It can be concluded on the basis of TD values that the amino acids in different feedstuffs are not directly additive since they are not available to the body to the same degree. In comparing casein and barley, for example, it can be seen that approximately 20% more amino acid from barley is required to equalize biologically the amino acids in casein. This situation is well known as different protein sources have specific experimentally determined digestibility coefficients. Since protein is made up of amino acids linked together it is inconsistent to estimate digestible protein but total amino acids. The solution to this problem, however, requires a knowledge of the TD of individual amino acids of which only relatively little information is available. Nevertheless, it must be considered more accurate to correct total amino acids with TD for total N in the corresponding material. This might be sufficiently precise and would furthermore relieve the necessity of working with different TD values for amino acids for each protein source. However, if the low TD for lysine applies to cereal varieties in general, this amino acid might be considered separately. For obvious reasons a correct estimate of available lysine in cereal proteins is of such importance that experiments should be performed with several varieties of cereals.

3. Biological value

In comparing the BV obtained on rats and pigs, it is of interest to consider the amino acid requirements of these two animal species. *The National Research Council* (*NRC* 1962, *NRC* 1968) has reported these requirements and *Rerat* (1971) has calculated the ratio between them;

Table 33. Requirement of essential and semiessential amino acids for growth of pigs expressed as a percentage of the requirement for rats

Tabel 33. Grisenes behov for essentielle og halvessentielle aminosyrer udtrykt i procent af rotternes behov

	Pig/Rat Ratio (%)		Pig/Rat Ratio (%)
Threonine	117	Methionine	109
Valine	93	Cystine	
Isoleucine	130	Histidine	79
Leucine	98	Lysine	101
Phenylalanine	72	Arginine	130
Tyrosine		Tryptophan	113

It appears from the NRC report that the requirement of lysine is very similar for rats and pigs whereas the requirement of sulphur-containing amino acids is 9% higher for pigs than for rats. This does not agree with the results of *Schiller & Ocio* (1963) who found a methionine supplement to casein to have a positive response in rats but not in pigs. Similar results are reported by *Brune et al.* (1968) and these workers emphasize that rats have higher requirements of sulphur-bearing amino acids than pigs, rendering rats unsuitable for the evaluation of pig diets. Thus *Brune et al.* (1968) obtained higher RC values in wheat, barley and oats when measured on pigs compared to rats. Furthermore similar RC values were obtained for these grains in experiments with rats, whereas different values were found with pigs. However, it should be mentioned that *Brune et al.* (1968) employed rats of 40 g and pigs of 17–20 kg. The stage of development of the experimental animals might be of importance when comparisons of this kind are made.

The results of *Brune et al.* (1968) differ from those obtained in the present work with cereal proteins in which the highest BV values were found with pigs. In work by *Nehring & Bock* (1961) higher BV values were also obtained in experiments with pigs compared to rats, although diets low in lysine and high in methionine gave the highest values in experiments with rats. This indicates a relatively higher requirement of sulphur-containing amino acids for rats than for pigs.

In order to provide further information regarding amino acid requirements, Table 34 shows the amino acid composition of total bodies of rats and pigs respectively (*Eggum* 1964).

The amino acid composition of the two animal species is very similar. However, the lysine and cystine contents in rats are somewhat higher than in pigs, whereas the opposite is the case with methionine. The relatively high cystine content in rats is surely due to the high concentration of this amino acid in hair (*Jørgensen & Eggum* 1971). Furthermore the figures in

Table 34. Amino acid composition of total bodies of rats and pigs
Tabel 34. Aminosyresammensætningen i hele rotter og grise

	Rat (80 g) (g/16gN)	Pig (30 kg) (g/16gN)
Lysine	6.17	5.67
Methionine	1.85	2.14
Cystine	1.48	0.98
Aspartic acid	8.57	8.89
Threonine	3.89	3.79
Serine	4.57	4.06
Glutamic acid	14.72	14.66
Glycine	9.35	10.89
Alanine	6.43	7.34
Valine	5.23	4.99
Isoleucine	3.71	3.67
Leucine	7.00	7.38
Tyrosine	2.81	2.71
Phenylalanine	4.52	3.99
Histidine	2.10	2.94
Arginine	6.33	6.53

Table 34 do not support the higher requirements of pigs for sulphur-containing amino acids reported by *NRC* (1968). In addition it is likely that smaller animals with a high metabolic rate have a higher requirement of methyl groups than larger animals (*Kleiber* 1961).

It is generally accepted that lysine is the limiting amino acid in cereal proteins and, as discussed above, positive responses are obtained when these products are supplemented with lysine. Consequently cereals showing the highest BV values might be expected to have the highest lysine contents, or more correctly the highest content of available lysine. The figures given in Table 17–22 do not, however, show this to be the case. Oats has the highest lysine content but rye has the highest BV. This might appear illogical if only the limiting amino acid is considered. Recent work by *Glem Hansen & Eggum* (1972a, 1972b) shows that the content of non-essential amino acids might affect BV. Consequently BV is not dependent on the first limiting amino acid alone, but on the whole aminogram. This makes it very difficult to predict an exact BV from the amino acid composition and imbalance is probably more common than hitherto appreciated.

As emphasized above, the BV values obtained for cereals are higher when measured on pigs than on rats. Furthermore the difference between these two animal species is higher the lower the lysine content. It is thus tempting to suggest that rats are more sensitive to lysine deficiency than pigs. However, this does not agree with the lysine requirements given by *NRC* (1968) and

in view of the work reviewed by *Young* (1970) regarding amino acid buffering capacity. The suitability of the experimental procedure used in present work with pigs can thus be questioned. Preliminary periods of 3 days and a balance period of 4 days were employed. Furthermore the pigs were fed a lysine-rich diet (Rød laktal) in the 5 days between periods and thus the lysine stores could be replenished. If this is the case then longer experimental periods for pigs will be required. However, it is difficult to induce younger pigs to eat cereal diets alone over longer periods.

The BV values obtained with concentrates fed to rats agree in general with the values which might be expected on the basis of the amino acid composition. The relatively low BV in casein will be discussed later. Thus it can be seen with both rats and pigs that the BV of fish meal is higher than that of casein despite a lower content of all essential amino acids. In soya bean meal a higher BV was recorded in pigs than in rats probably due to a lower requirement of sulphur-containing amino acids for pigs. This is also indicated by the higher BV of meat and bone scraps than of both groundnut and sunflower seed meals despite the fact that the latter show higher contents of sulphur-bearing amino acids. Since the concentrated feeds were fed at different N concentrations to rats and pigs respectively, a direct comparison of BV cannot be made in these diets.

4. Net protein utilization

Since NPU is a derived factor (TD · BV/100) no biological comment can be given. This figure only indicates the actual retention in the organism. It appears from the present work with rats that NPU in barley, oats and rye is approximately 60 and in wheat and maize almost 50. The lowest NPU (44.3) was obtained in sorghum, i. e., more than half of the protein consumed is excreted. The corresponding NPU values obtained with pigs are all higher since BV is higher and TD approximately the same in both animal species.

Of the concentrated feeds fed to rats, casein and fish meal showed the highest NPU values of approximately 70%, followed by sunflower seed meal with 64.9 and soya bean meal and groundnut meal with about 56, while meat and bone scraps showed an NPU of only 42.3. The NPU values of the mixtures are all high, 68.5 and 66.5 for the combination dehulled oats with skim milk powder and soya bean meal respectively, while the NPU of the milk substitute was 76.7, i. e. an excellent quality.

It should be emphasized here that proteins having the same NPU values do not necessarily have the same value in a mixture. Soya bean meal and groundnut meal, for example, both have an NPU of 56 but it is well known that protein from soya bean rich in lysine and threonine has a much higher complementary effect in a mixture than groundnut meal, particularly when combined with cereals.

5. Utilizable nitrogen

UN is also a derived factor, the calculation including both a quantitative (N in % of dry matter) and a qualitative (NPU) factor. In the experiments with rats an UN value of approximately 1 was obtained for barley, oats, wheat and sorghum and values of 0.86 and 0.82 for rye and maize respectively. The low values for rye and maize are particularly due to a low N content. Casein and fish meal both show very high UN values (10.92 and 9.04 respectively), i.e., fish meal contains approximately 9 times more utilizable nitrogen than cereals. Soya bean meal and groundnut meal have almost the same UN, 4.61 and 4.75 respectively, while sunflower seed meal has the somewhat lower value of 4.25 and meat and bone scraps only 3.73%.

From this discussion it would appear that UN is particularly low in the cereals. It would be of global interest if these values could be increased. At present much attention is being given by cereal breeders to the increase of both protein quality and quantity. Such work has already succeeded in giving UN values of 2 or even more in barley, maize and wheat (*Eggum* 1971).

R. Comparison of results obtained with rats and pigs

For comparison of the results obtained with rats and pigs respectively, regression equations and correlation coefficients were calculated between the individual TD values of all fifteen diets, whereas in the case of BV only the cereal diets were used in these calculations. Only the cereal diets had the same N concentration for both rats and pigs and are thus directly comparable. However, since the observations cover only a narrow region of the regression line it is difficult to obtain high correlation coefficients. The comparisons between rats and pigs of TD for lysine and total nitrogen in the 15 diets are illustrated in Figures 10 and 11 respectively, while the comparisons between the BV values in the 6 cereal diets are shown in Figure 12.

A t-test was carried out to detect significant differences between the results obtained with rats and pigs. These results are shown in Table 35 together with the regression equations and correlation coefficients.

In spite of the narrow ranges of observations covered by the regression lines the r-values are high, with several above 0.90. The regression coefficients are all significantly different from zero, a t-test showing $P < 0.001$. A low correlation coefficient was found only in the case of serine (0.61), the reason for which is not clear.

The results of t-tests on the differences between corresponding values for rats and pigs showed that the TD values obtained with rats were not significantly different from those obtained with pigs. Thus the results obtained with rats in this type of experiment would also appear to be applicable to pigs.

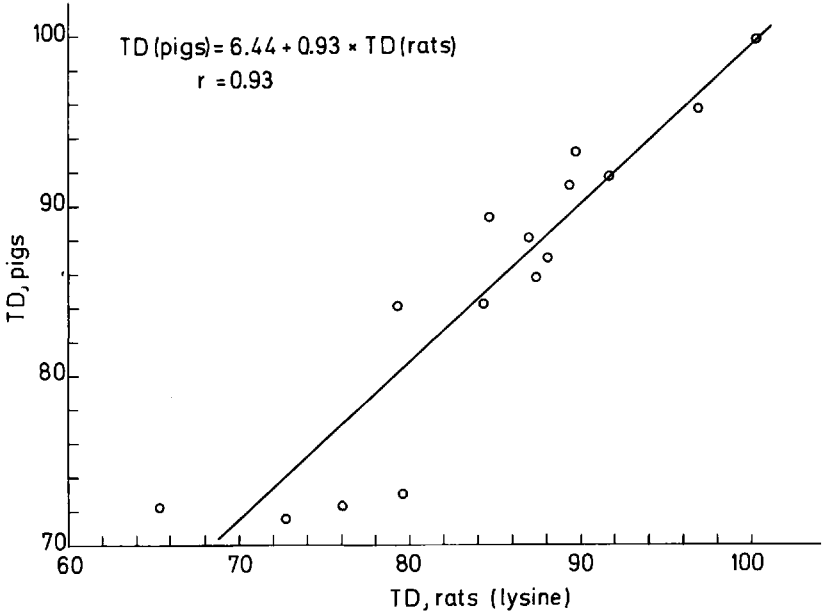

Figure 10. Comparison of TD (true digestibility) values of lysine obtained in 15 diets with rats and pigs respectively

Figur 10. Sammenligning af SF (sand fordøjelighed) -værdier for lysin i 15 fodermidler målt på henholdsvis rotter og grise

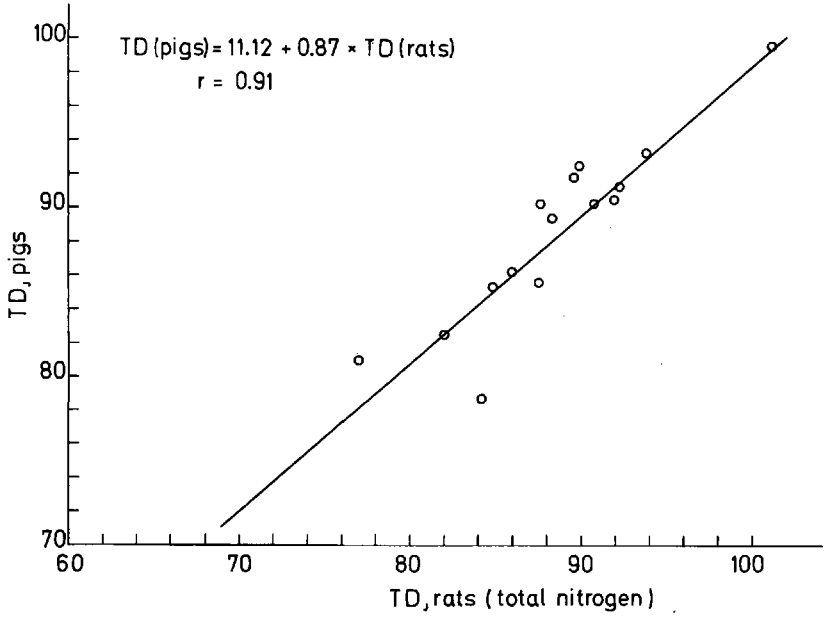

Figure 11. Comparison of TD (true digestibility) values af total N obtained in 15 diets with rats and pigs respectively

Figur 11. Sammenligning af SF (sand fordøjelighed) -værdier for total-N i 15 fodermidler målt på henholdsvis rotter og grise

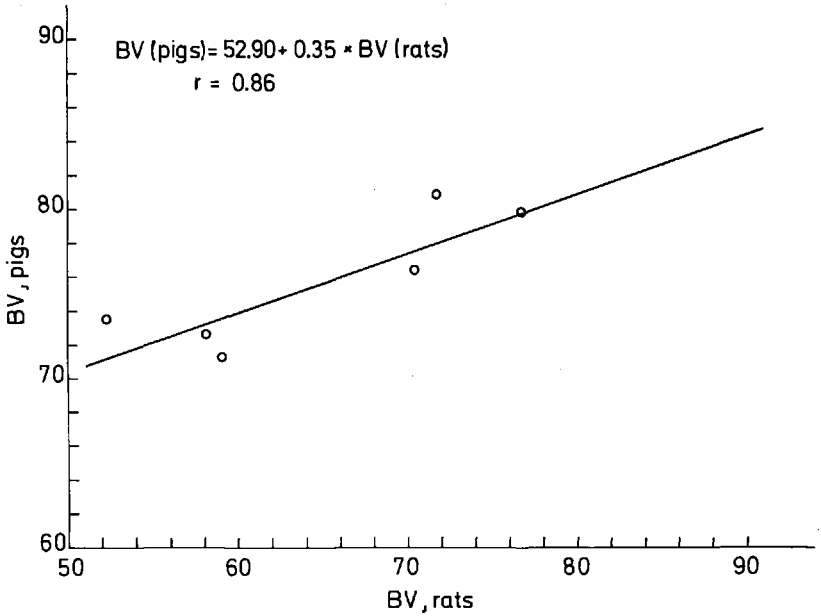

Figure 12. Comparison of BV (biological value) values obtained in 6 grain diets with rats and pigs respectively

Figur 12. Sammenligning af BV (biologisk værdi) -værdier i 6 kornarter målt på henholdsvis rotter og grise

Poppe & Meier (1971b), however, obtained some higher TD values in experiments with pigs compared to data obtained in experiments with rats. As previously discussed, the relationship between BV values is less pronounced than in the case of TD values. Nevertheless, the relative values may be usefull and BV is thus considered to warrant more attention. It is also assumed that the high correlation between the TD values obtained with rats and pigs indicates a negligible microbial effect on these values. This is based on the assumption that any severe microbial activity will reduce the correlation between observations made on these two animal species. The results given in Table 35 thus confirm the applicability of the faecal analysis method for the estimation of the availability of the individual amino acids in feedstuffs.

On the basis of the results described in the present chapter, studies were continued using rats alone of the factors affecting protein utilization. It was considered probable that certain of the results thus obtained might also be of value for our domestic animals.

Table 35. Regression equations and correlation coefficients between the individual biological criteria obtained from experiments with 15 diets fed to rats and pigs respectively
Tabel 35. Regressionsligninger og korrelationskoefficienter mellem de enkelte biologiske kriterier fundet i forsøg med 15 fodermidler til henholdsvis rotter og grise

			r	s	s_b	t[1])
Lysine .....TD (pigs)	= 6.44 + 0.93	· TD (rats)	0.93	3.57	0.11	0.538
Methionine .....TD (pigs)	= 7.27 + 0.92	· TD (rats)	0.89	3.69	0.13	0.423
Cystine .....TD (pigs)	= 11.96 + 0.86	· TD (rats)	0.90	2.99	0.12	0.477
Aspartic acid ...TD (pigs)	= 5.58 + 0.95	· TD (rats)	0.93	2.67	0.10	1.352
Threonine .....TD (pigs)	= 6.17 + 0.94	· TD (rats)	0.95	2.37	0.09	1.232
Serine .....TD (pigs)	= 40.08 + 0.55	· TD (rats)	0.61	3.36	0.20	2.786**)
Glutamic acid ...TD (pigs)	= 34.53 + 0.63	· TD (rats)	0.86	1.99	0.11	0.660
Glycine .....TD (pigs)	= 12.74 + 0.85	· TD (rats)	0.90	2.82	0.12	0.607
Alanine .....TD (pigs)	= 1.00 + 0.98	· TD (rats)	0.92	3.21	0.12	1.365
Valine .....TD (pigs)	= –8.67 + 1.09	· TD (rats)	0.94	2.34	0.11	0.503
Isoleucine .....TD (pigs)	= 17.97 + 0.81	· TD (rats)	0.83	4.06	0.15	1.391
Leucine .....TD (pigs)	= 5.73 + 0.93	· TD (rats)	0.92	2.18	0.11	0.872
Tyrosine.....TD (pigs)	= 24.27 + 0.74	· TD (rats)	0.84	3.41	0.13	1.128
Phenylalanine ...TD (pigs)	= 17.33 + 0.80	· TD (rats)	0.84	3.31	0.15	0.995
Histidine .....TD (pigs)	= 24.83 + 0.72	· TD (rats)	0.76	3.16	0.17	1.855
Arginine .....TD (pigs)	= 38.38 + 0.59	· TD (rats)	0.85	2.04	0.10	0.120
TD Total nitrogen (pigs)	= 11.12 + 0.87	· TD (rats)	0.91	2.30	0.11	0.178
BV Biological value (pigs)	= 52.90 + 0.35	· BV (rats)	0.86	2.24	0.10	4.130**)

[1]) t = t-values on the differences
**) $P < 0.01$

CHAPTER VIII

The influence of dietary protein level on protein utilization

A. General discussion

It is generally agreed that protein utilization is higher at low protein levels than at high levels. This relationship, demonstrated by *Mitchell* (1924b), is evident when animals are fed excessive protein since the organism is forced to use protein as energy source and the corresponding nitrogen is discarded into the urine. The situation may not be quite so evident when the animals are offered sufficient energy from N-free components.

Thus *Forbes et al.* (1958) found that increasing protein levels from 4 to 29% with egg, casein and groundnut meal led to a direct negative linear relationship between BV and protein content with the exception of data from 4 to 8% egg protein. In this interval BV was almost constant and approximated 100%. In experiments with casein to adult rats *Henry & Kon* (1957) found a higher BV at 4% protein in the diet than at 8%. Furthermore, they found higher values in the same animals when young than when fully grown. Similar results were obtained by *Nehring & Haesler* (1954) and *Nehring & Bock* (1961) who found that a protein content below 10% produced higher BV values than a protein content above 10%. *Morrison et al.* (1963) stated that the decrease in protein utilization is proportional to the logarithm of the protein content in the diet.

Miller & Payne (1961) found in experiments with rats that a negative correlation exists between NPU and calories from the protein fraction. This was shown by increasing the amounts of protein from beef, casein and wheat gluten from 10 to 40% in the diet. The regression coefficient for beef was –1.68, for casein –1.39 and for wheat gluten –0.79. It would thus appear that the higher the protein quality the more rapidly the NPU value decreases with increasing calories in the diet from the protein fraction. They found that when 10% of the calories were derived from the protein, the NPU for beef was 73, for casein 62 and for wheat gluten 36. The differences between the NPU values were considerably less pronounced when 40% of the calories were obtained from protein; NPU values of 25, 22 and 19 were recorded for beef, casein and wheat gluten respectively. These results clearly demonstrate the way in which an excessively high protein content in experimental diets can conceal any real differences in quality.

True digestibility is generally considered to be unaffected by the protein level in the diet. *Henry & Kon* (1957) found very small differences in experiments with rats, whereas *Wiesemüller & Poppe* (1969b) found no differences in the digestibility of casein after increasing the content in diets fed to pigs.

These results are in agreement with the results obtained by *Allison et al.* (1946), *Mitchell* (1948), *Forbes et al.* (1958), *Njaa* (1959) and *Lehmann & Hock* (1968). Furthermore, these investigations showed that TD is also independent of feed intake and body weight of the animals.

As emphasized by *Njaa* (1963) a sharp distinction must be made between the concepts of true and apparent protein digestibility. The latter concept is meaningful only under strictly standardized conditions, whereas the former can be regarded as a characteristic of the particular protein slurce regardless of the dietary conditions under which it is given. *Dammers* (1964) and *Just Nielsen* (1970) have shown the same relationship between AD and protein level in experiments with pigs.

It would thus appear that the protein level in the experimental diet is generally considered to influence AD and BV but not TD. According to *Gouwens* (1966) TD of the essential amino acids is also independent of the protein level in the diet.

B. Present investigations

In order to investigate the effect of dietary protein level on protein utilization, experiments were carried out with rats fed increasing amounts of protein. Two protein sources – casein supplemented with 1% DL-methionine and

Table 36. The influence of protein level on protein utilization. Casein + 1% DL-methionine as protein source

Tabel 36. Indflydelsen af proteinniveauet på proteinets udnyttelse. Kasein + 1% DL-methionin som proteinkilde

N/day/rat (mg)	Protein in dry matter (%)	AD (%)	TD (%)	»BV« (%)	NPU (%)
47	5.39	74.2	98.4	83.2	81.9
87	6.42	76.5	99.1	87.5	86.7
97	7.65	82.8	99.4	89.2	88.7
137	9.30	85.5	99.4	84.8	84.3
131	9.44	85.7	99.7	81.3	81.0
159	9.97	85.5	98.7	82.9	81.8
175	11.47	86.9	98.1	79.6	78.1
194	12.81	89.0	98.9	77.4	76.6
206	14.14	90.5	95.5	75.7	75.4
228	15.20	90.8	99.4	71.7	71.3
224	16.66	91.2	99.2	71.2	70.6
221	18.01	92.5	99.6	(– lost –)	
288	19.81	91.4	97.7	61.6	60.2
332	22.38	92.5	98.1	57.8	56.7
364	25.31	93.3	98.3	53.0	52.2
375	27.93	93.3	97.8	44.8	43.8
396	30.49	94.8	99.0	44.6	44.2

soya bean meal – were employed. The casein + methionine was fed at 17 different protein levels, while soya bean meal was fed at only 9 different levels. The results with casein are shown in Table 36. AD, TD, »BV« and NPU were used as criteria.

As can be seen from Table 36, the TD values were found to be independent of the protein level in the diets and approached 100. The AD values, however, were considerably affected and increased from 74.2 at the lowest protein intake to 94.8 at the highest level. The effect on the »BV« values was opposite, the lowest values being obtained at the highest protein consumption. »BV« was approximately 90 at the lower protein levels and decreased to 44.6 at the highest protein intake. As in the experiments with egg protein carried out by *Forbes et al.* (1958), the »BV« values shown in Table 36 increased and reached a maximum at approximately 8% protein and then decreased linearly. The data of Table 36 are illustrated in Figure 13.

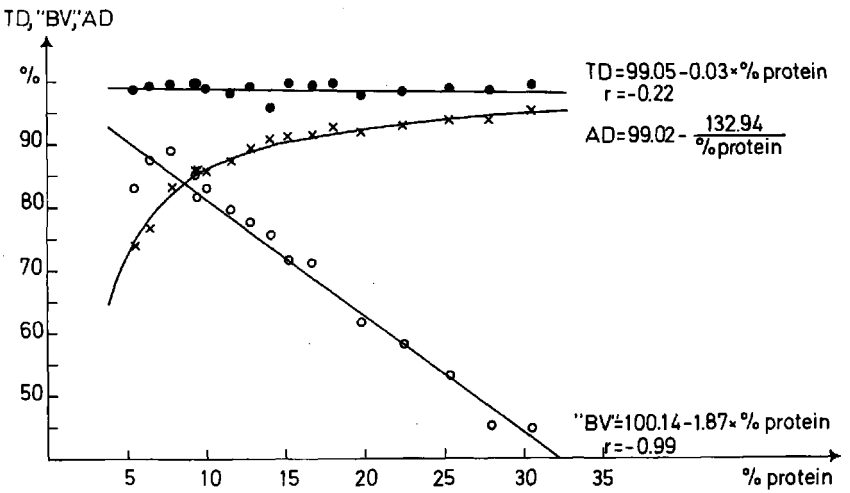

Figure 13. AD (apparent digestibility), TD (true digestibility) and »BV« (»biological value«) of casein in relation to the protein content in the diet

Figur 13. TF (tilsyneladende fordøjelighed), SF (sand fordøjelighed) og »BV« (»biologisk værdi«) i kasein sat i relation til foderets proteinindhold

A calculation of the regression between protein level and TD, »BV« and AD values respectively gave the following equations:

Equation 18: $TD = 99.05 - 0.03 \cdot \%\ \text{protein}$
$s = 1.03;\ s_b = 0.03;\ r = -0.22$

Equation 19: $»BV« = 100.14 - 1.87 \cdot \%\ \text{protein}$
$s = 2.56;\ s_b = 0.08;\ r = -0.99$

Equation 20: $AD = 99.02 - \frac{132.94}{\%\ \text{protein}}$
$s = 0.84;\ s_b = 4.84;\ r = 0.99$

As expected, the regression coefficient for TD showed no significant relationship between this value and the level of protein. »BV«, however, decreased significantly ($P < 0.001$) as the protein intake increased. The equation for AD is calculated according to the model $y = a - \frac{b}{x}$ and clearly demonstrates the effect of protein level on AD values.

As mentioned above, additional experiments with increasing dietary protein levels from soya bean meal were carried out. The results are shown in Table 37.

Table 37. The influence of protein level on protein utilization. Soya bean meal as protein source
Tabel 37. Indflydelsen af proteinniveauet på proteinets udnyttelse. Sojaskrå som proteinkilde

N/day/rat (mg)	Protein in dry matter (%)	AD (%)	TD (%)	»BV« (%)	NPU (%)
97	6.36	65.0	84.9	81.4	69.0
142	9.40	75.1	87.2	75.9	66.2
203	12.60	75.8	86.6	70.4	60.9
252	15.59	78.6	86.7	64.9	56.3
295	18.85	81.2	87.9	61.8	54.3
352	22.02	82.7	88.5	56.9	50.2
408	25.35	81.6	86.6	52.7	45.6
447	28.19	83.3	87.8	51.3	45.1
491	31.14	82.1	86.2	46.3	39.9

These experiments show approximately the same results as those with casein, namely increasing AD values, unaffected TD values and decreasing »BV« values with increasing protein levels. The data of Table 37 are shown graphically in Figure 14.

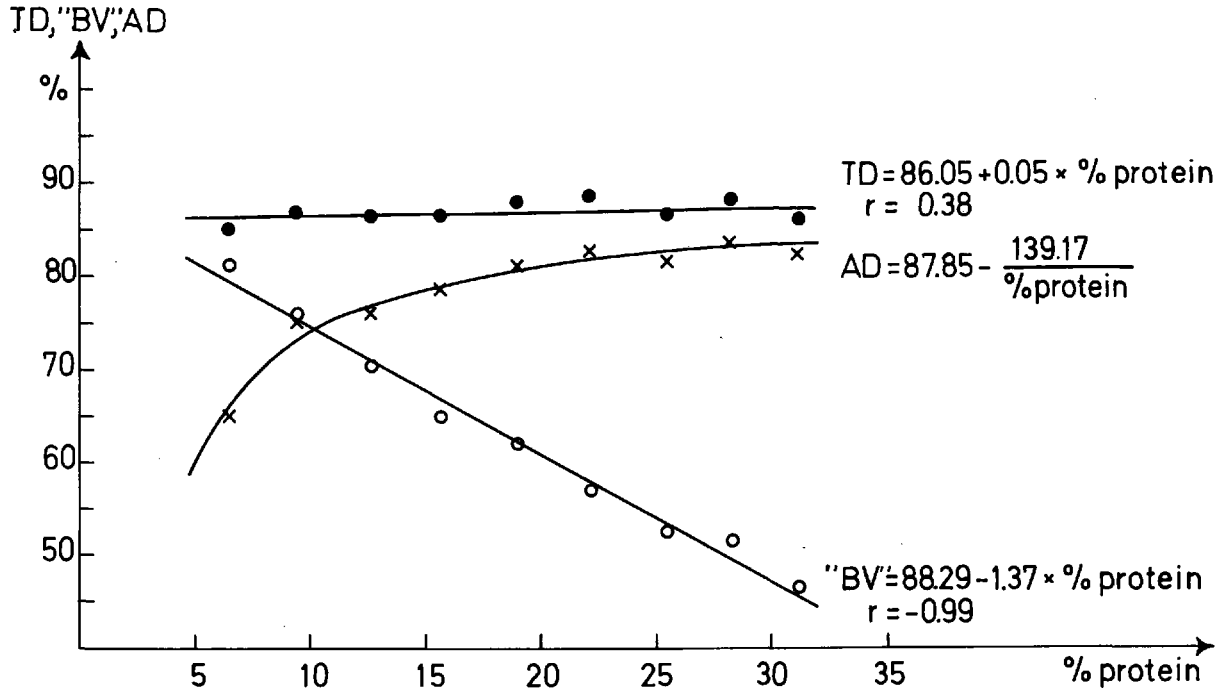

Figure 14. AD (apparent digestibility), TD (true digestibility) and »BV« (»biological value«) of soya bean meal in relation to the protein content in the diet

Figur 14. TF (tilsyneladende fordøjelighed), SF (sand fordøjelighed) og »BV« (»biologisk værdi«) i sojaskrå sat i relation til foderets proteinindhold

A calculation of regression between protein level and TD, »BV« and AD values respectively gave the following equations:

Equation 21: $TD = 86.05 + 0.05 \cdot \%\ \text{protein}$
$s = 1.06;\ s_b = 0.04;\ r = 0.38$

Equation 22: $»BV« = 88.29 - 1.37 \cdot \%\ \text{protein}$
$s = 1.40;\ s_b = 0.06;\ r = -0.99$

Equation 23: $AD = 87.85 - \frac{139.17}{\%\ \text{protein}}$
$s = 1.22;\ s_b = 10.52;\ r = 0.98$

As for casein there was no significant correlation between TD and dietary protein level, whereas »BV« decreased significantly ($P < 0.001$) with protein intake. AD, however, increased rapidly with an increase in protein intake and finally reached a plateau at about 20% protein in dry matter. As is seen in Figure 13 this was also the case with casein.

C. Discussion

As can be seen from Figure 13 and 14, AD is markedly influenced by the protein level in the diet. AD values can thus only be compared under standardized conditions, i.e., the protein intake must at least be maintained at a constant level. Fluctuations in the protein level can help to explain the discrepancies in the literature between AD values determined on the same protein source.

The increase in AD with increasing protein intake was greatest at the lower protein levels; at the highest levels the increase was negligible. This effect is due to the assumed constant metabolic nitrogen excretion which constitutes a greater portion of the total N excretion at the lower protein levels.

As previously discussed, TD is not affected by the protein level, a fact also demonstrated by the present experiments with casein and soya bean meal as protein sources. Metabolic N would thus appear to be independent of the N content in the diet. Work by *Twombly & Meyer* (1961), however, appeared to show that metabolic N increases with the level of protein in the diet.

»BV« and NPU are generally found to be highly sensitive to changes in dietary protein concentration. The gradient of the regression line for »BV« appears to differ for the two protein sources in Figure 13 and 14. Regression coefficients of –1.87 and –1.37 were found for casein + 1% methionine and soya bean meal respectively. Hence an increase in protein content resulted in a more rapid decrease in »BV« in the former source than in the latter.

Similar results were reported by *Miller & Payne* (1961) who found protein sources with high NPU to decrease more rapidly than those with lower NPU when the protein content in the diet increased.

This difference in rate of decrease in »BV« is probably due to the fact that protein sources with high BV usually also possess a high TD. Thus relatively more nitrogen is offered to the organism from proteins with high NPU compared to proteins with lower NPU even if the protein content in the diet is the same. The higher BV together with the higher absolute nitrogen absorbed may result in a more rapid decrease in the utilization of casein + 1% methionine with increasing protein concentration than in the case of soya bean meal.

At low dietary protein levels the absorbed protein might be completely utilized. An increase in the protein supply, however, will generally result in an increase in the proportion of protein used for fat synthesis. The quality of the protein (BV) is of importance only for that fraction used for protein synthesis. Thus an increase in dietary protein will lead to a gradual reduction in the degree to which protein quality influences the regression line. Proteins with high BV will therefore necessarily have higher negative regression coefficients than proteins with lower BV.

The results presented in Figure 13 and 14 clearly show that a high protein content in the experimental diet can obscure differences in BV due to the fact that the various proteins show differing responses to the protein level in the diet.

CHAPTER IX

The influence of dietary energy level on protein utilization

A. General discussion

It is generally accepted when evaluating protein quality that the experimental animals must be provided with sufficient energy from an N-free fraction. In this way protein will not be employed as a source of energy and consequently can be used exclusively for protein synthesis, i.e., maintenance or maintenance and growth. However, there is some discussion as to whether a high energy supply in the diet can have a protein-saving effect.

In experiments with rats *Forbes & Yohe* (1954) have illustrated the influence of energy consumption on biological value. In this work two protein sources were employed, soya bean meal and blood fibrine with and without extra methionine respectively. The diets were adjusted to 10% protein and fed at 4.0, 6.0 and 8.0 g pr. animal. The results showed that at the lowest feed consumption (4.0 g) the biological value was lower than at 6.0 and 8.0 g respectively per day per animal. The animals were obviously forced to use protein as energy source at the lowest feed consumption and the corresponding nitrogen was excreted with the urine. These investigations demonstrate the importance of a sufficient energy supply when evaluating protein quality.

In experiments with rats *Yoshida et al.* (1957) investigated the effect on nitrogen retention of varying the ratio between calories from protein and calories from the N-free fraction by feeding 10% protein together with 0, 10 and 30% fat. The results showed, however, that increasing fat content did not affect nitrogen retention. *Lowrey et al.* (1963) also found that increasing the energy content in diets to pigs had no effect on nitrogen balance or apparent nitrogen digestibility. *Morrison* (1963) investigated the influence of the fat level in diets on protein utilization expressed by protein efficiency ratio (PER) after feeding 10 and 20% maize oil, lard and margarine respectively. The PER values, however, were quite unaffected by the two fat levels. In a comprehensive study *Metta & Mitchell* (1956) came to a similar conclusion, but suggested that an increase in fat might cause an increase in ketone bodies. The kidneys may produce more ammonia to neutralize these acids and consequently an increase in tissue catabolism would occur. This does not appear to be the case, however, since *Metta & Mitchell* (1956) found no difference in protein utilization after substituting fat for carbohydrates in the range between 5 and 33%. The fat level had no effect on AD, TD or BV.

Contrary to the experiments outlined above, *Likuski et al.* (1961) found that the nitrogen retained as a percentage of nitrogen absorbed increased with the dietary energy level in rats, whereas the opposite applied to pigs.

Other workers (*Munro* 1964, *Homb & Lysö* 1964, *Wiesemüller & Poppe* 1969c), however, reported a positive effect on protein utilization in pigs due to high energy levels.

Munro (1951) concluded in his review article that »1) the addition of extra energy to a complete diet usually leads to a positive nitrogen balance, 2) this effect is related to the energy supplied, 3) energy from fat and carbohydrates causes the same increase in nitrogen retention, 4) the extra supply of fat and carbohydrates does not necessarily have to be given together with the protein to result in nitrogen retention and 5) extra energy may have an influence up to 15 days in human beings.«

From this discussion it would appear that opinions are somewhat divergent regarding the influence of dietary energy on protein utilization. For further illustration of this problem the results of investigations carried out by the present author are described in the following section.

B. Present investigations

Experiments are carried out with rats fed diets containing increasing energy concentrations and a constant protein level (9.4% of dry matter). In increasing the energy concentration, carbohydrates were replaced by fat (90% lard + 10% soya bean oil). The N-free diet contained 4.5% soya bean oil. Casein supplemented with 1% DL-methionine was used as nitrogen source.

Table 38. The influence of dietary energy level on protein utilization
Tabel 38. Energiniveauets indflydelse på proteinets udnyttelse

Fat supplied in the diet (%)	TD (%)	BV (%)	NPU (%)
0	97.3	88.2	85.8
2	96.2	87.6	84.2
4	96.9	90.0	87.2
6	97.3	86.9	84.5
8	97.4	90.9	88.6
10	97.6	88.9	86.8
12	99.0	88.7	87.8
14	97.4	86.1	83.9

The inclusion of extra fat in the diets caused lumping and resulted in some feed loss. This is probably the reason for the somewhat random variation in the BV values. Nevertheless the results do not indicate any positive effect on the criteria studied due to increase in energy content of the diet. This conclusion is in agreement with most of the experiments preciously described which show high energy levels in the diets to have no effect on protein utilization.

In order to determine possible effects on protein quality, further experiments were carried out with fat supplements to diets of different biological value. The protein sources in diets of high BV were herring meal, soya bean meal and a cereal mixture, whereas meat and bone scraps, sunflower meal and a cereal mixture made up the protein sources in the low BV diets.

Table 39. The influence of dietary energy level on protein utilization in diets of high and low BV

Tabel 39. Indflydelsen af energiniveauet på proteinets udnyttelse i foder med høj og lav BV

Fat supplied in the diet (%)	Biological value	TD (%)	BV (%)	NPU (%)
0	High	88.7	80.8	71.8
6	»	89.0	79.6	70.8
10	»	90.1	79.5	71.6
0	Low	85.9	65.6	56.3
6	»	85.6	66.7	57.1
10	»	84.8	65.1	55.4

The results in Table 39 are similar to those obtained with casein, i.e., the extra energy supply has no influence on protein utilization irrespective of the protein quality. The diets shown in Table 39 were also employed in experiments with pigs (*Thorbek & Eggum* 1969) and similar results were obtained.

It can thus be concluded that there is no indication of any specific protein-saving effect due to high energy contents from fat.

CHAPTER X

The influence of dietary crude fibre on protein utilization

A. General discussion

It is generally assumed that an increase in crude fibre in diets has a negative effect on digestibility, particularly on the digestibility of the nitrogen-free extracts and proteins.

Thus *Fingerling et al.* (1914a, 1914b) found in experiments with pigs that cellulose from straw gave a slight depression in digestibility of the N-containing sources. *Breirem et al.* (1943), however, reported a pronounced negative effect on the apparent protein digestibility when pigs were fed diets with various types of cellulose. On average this negative effect amounted to 54.5 ± 4.1 g digestible protein per kg dry matter in cellulose. Similar values were found by *Nordfeldt* (1942) and by *Woodman & Evans* (1947) and *Evans & Maguire* (1956). *Horszczaruk* (1962) demonstrated that feeding increasing amount of fibre to pigs had a negative influence on the digestibility of dry matter, crude protein and N-free extracts. Similar values were recorded by *Thomke* (1961) in digestion experiments with sheep, pigs and chickens fed oats with 9.0, 12.0 and 15.0% crude fibre respectively. Recent investigations by *Just Nielsen* (1970) in pigs showed a similar negative effect due to crude fibre as did *Meyer* (1956) in experiments with rats.

Zorita & Schobinger (1958) suggested that lignin gives rise to an increase in metabolic N secretion and hence a decrease in apparent protein digestibility. They were, however, unable to determine whether this was due to physical, physico-chemical or chemical conditions alone. *Whiting & Bezeau* (1957) also found an increase in metabolic N with increasing amounts of crude fibre from cellulose in diets for pigs. In addition to the apparent digestibility, true digestibility was also found to decrease. The biological value, however, appeared to be independent of the cellulose content in the diet. *Mangold & Behm* (1955) reported a similar increase in metabolic N with increasing concentrations of crude fibre in diets for rats, rabbits and pigs.

Madsen (1963) showed in digestion experiments with pigs that the digestion of crude protein decreased by 0.91% for each 1% increase in crude fibre content at a constant protein level. These values were determined on the basis of 39 feedstuffs in regression analyses. *Madsen* (1963), however, drew attention to the fact that such calculations carried out on a large but varied material might be of doubtful value.

These considerations indicate certain problems in experimental technique concerning the influence of crude fibre on the digestibility of protein. If, for example, the crude fibre content is increased concurrently with changes

in the proportions of the nitrogen sources, a decrease in the protein digestibility could equally well be attributed to the change in proteins. Since such comparisons will not involve diets of identical composition, this procedure cannot provide definite information on the effect of crude fibre on protein utilization. Hence *Bønsdorff Petersen* (1971) ahowed that the changes in protein digestibility obtained by increasing crude fibre content to chickens sould be explained on the basis of the differences in true digestibility of the proteins used.

Experiments by *Eggum* (1970a, 2970b) indicate that even barley protein can be digested differently from one sample to another. *Braham & Bressani* (1969) found in experiments with rats that neither crude fibre (celluflour) nor fat had any effect on the NPU of soya bean meal. The animals were fed diets containing 0.8 to 10.0% crude fibre or 1.7 to 10.0% fat. *Dammers* (1964) also found that additional cellulose in diets for pigs had no influence on the digestibility of protein. Similar results were reported by *Gouwens* (1966). Diets containing 7, 14 and 21% crude fibre were fed to pigs. No significant differences in nitrogen balance or faecal nitrogen excretion were observed among the levels of dietary fibre. The faecal amino acid pattern appeared to be constant at all dietary fibre levels and no relationship was established between dietary fibre level and total faecal excretion of individual amino acids.

B. Present investigations

1. Protein sources with different crude fibre contents

The differing opinions concerning the influence of crude fibre have led the majority of workers to adjust the fibre concentration to an approximately constant level in experimental diets when evaluating protein quality. However, a calculation carried out on material of *Eggum* (1968a) has shown that protein sources rich in crude fibre had lower TD values than proteins with low crude fibre content, even when the crude fibre content in the experimental diets was adjusted to the same concentration. These results are shown graphically in Figure 15 with 22 feedstuffs. The crude fibre content of the protein sources varied from 0.2 to 21.0% whereas the true digestibility of the proteins varied from 71.0 to 99.0%.

A calculation of the regression between the TD and crude fibre values in the 22 samples gave the following equation:

Equation 24: $TD = 94.02 - 1.10 \cdot \%$ crude fibre
$s = 3.97$; $s_b = 0.13$; $r = -0.88$

A t-test showed the regression coefficient to be significantly different from zero ($P < 0.001$). Thus the equation shows that the true digestibility of the

proteins decreases as the crude fibre content in the protein sources increases. This investigation appears to indicate that the protein in feedstuffs high in crude fibre has a lower digestibility than the protein in feedstuffs low in crude fibre. It should be emphasized that this does not imply that the crude fibre has a negative effect on the protein digestibility. This will be discussed in the following section.

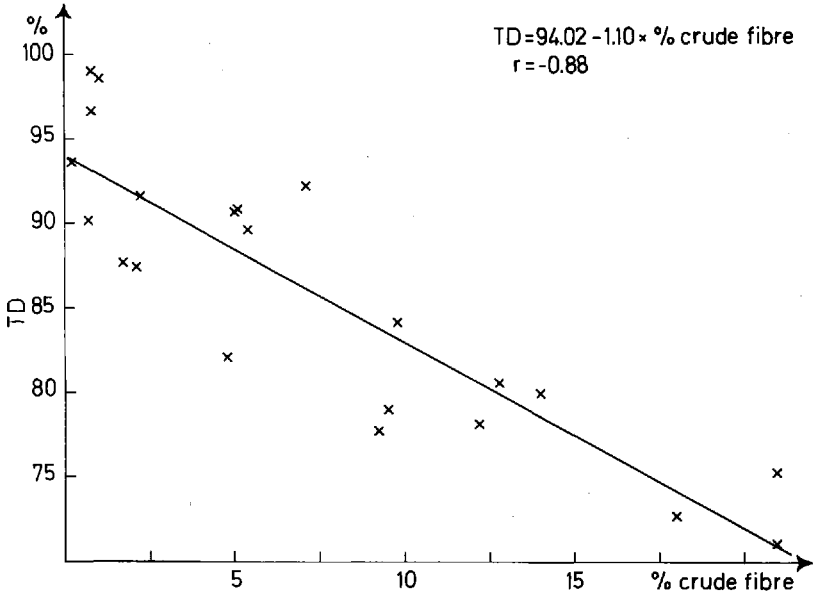

Figure 15. TD (true digestibility) in relation to crude fibre content in the protein sources
Figur 15. SF (sand fordøjelighed) i relation til træstofindholdet i proteinkilderne

2. The influence of dietary crude fibre on metabolic nitrogen

It would appear that an increase in crude fibre (*Zorita & Schobinger* 1958) can affect the metabolic N secretion, i.e., this value will increase and consequently the apparent digestibility will decrease.

To illustrate this relationship increasing amounts of »Whatman« cellulose powder (72.7% crude fibre) were fed as replacements for N-free diets in experiments with rats. In these experiments the same protein source (casein supplemented with 1% DL-methionine) was employed and the protein level was kept constant. In the calculations metabolic N is assumed to be dependent upon dry matter consumption and independent of crude fibre content. If the

crude fibre influences the metabolic N, this method of calculation should result in a decrease or an increase in TD values with increasing crude fibre content.

A total of 15 levels of cellulose powder were investigated, increasing from 2 to 30% of dietary dry matter. TD, BV and NPU were determined and the results are shown in Table 40.

Table 40. The influence of dietary crude fibre on protein utilization
Tabel 40. Træstoffets indflydelse på proteinets udnyttelse

Cellulose powder in dry matter (%)	TD (%)	BV (%)	NPU (%)
0	100.0	82.9	82.9
2	99.1	82.7	81.9
4	99.5	83.8	83.3
6	99.4	83.9	83.4
8	100.1	83.0	83.1
10	99.9	81.5	81.4
12	100.3	82.4	82.7
14	100.2	82.1	82.3
16	99.7	81.1	81.8
18	100.9	81.5	82.2
20	100.6	81.2	81.7
22	100.6	79.3	79.8
24	97.6	83.2	81.2
26	98.8	80.7	79.7
28	99.0	77.3	76.5
30	99.2	77.9	77.3

As shown in Table 40, an increase in crude fibre from N-free cellulose powder was not found to influence the TD values. This would suggest that crude fibre has no influence on the secretion of metabolic N and consequently no effect on the apparent digestibility. Similarly *Bønsdorff Petersen* (1971) found in experiments with chickens that AD was independent of increasing amounts of cellulose powder in the diet.

In accordance with experiments by *Meyer* (1956) the biological value also appears to be unaffected by fibre content; the BV was very much the same at the different cellulose concentrations, although with a tendency for lower BV values at the highest fibre levels. This might be due to the fact that the animals were forced to use protein as energy source because of the low content of metabolizable energy in cellulose.

On the basis of these results it is concluded that crude fibre does not directly affect protein utilization. Feedstuffs high in crude fibre contain nitrogen which is not digested with the same efficiency as the nitrogen in feedstuffs with a lower crude fibre content. This is, however, not necessarily due to the crude fibre level as such.

CHAPTER XI

The influence of lactose on protein utilization

A. General discussion

The response of an animal to a particular protein has been shown to depend upon the nature of the carbohydrate in the meal (*Guggenheim et al.* 1960, *Dahlquist & Thomson* 1964). One of the most important factors associated with lactose is that it appears to influence gastro-intestinal mobility. *Ficher & Sutton* (1949) reported that lactose can give rise to changes in pH sufficiently large to stimulate the intestinal musculature. This has also been demonstrated by several investigations carried out with chicken (*Beach & Davis* 1925, *Beach* 1925, *Kline et al.* 1932, *Ashcraft* 1933). *Maner et al.* (1962) reported that differences in gastric pH and rate of food passage may be factors involved in the efficiency of protein utilization by very young pigs.

Several workers have observed that complex carbohydrates are superior to simple sugars in tests such as protein efficiency ratio and growth rate (*Henderson et al.* 1947, *Hankes et al.* 1948, *Harper & Katayama* 1953, *Chang* 1962). This has been attributed to a slower passage of the digesta with more complete amino acid liberation (*Register & Petterson* 1958), although *Chang* (1962) found no improved digestibility and *Spivey et al.* (1958) observed no improved release or absorption of amino acids. *Buraczewski et al.* (1971) found in rats that the efficiency of certain carbohydrates in delaying stomach emptying was in the following ascending order: maize starch, maize dextrine, glucose, soluble starch, sucrose and lactose. Too rapid a passage through the intestine may impair not only the digestion of the dietary protein but also that of the metabolic secretions, leading to severe nitrogen losses (*Harper et al.* 1952).

Wilbur et al. (1960) demonstrated that the gain and efficiency of feed utilization in pigs was far superior when lactose was included in the diet than when maize starch was employed. All rectal organisms, with the exception of the obligate anaerobes and lactobacilli, were less numerous when lactose was fed. In each section of the intestinal tract coliforms, streptococci, staphylococci, moulds and yeast were less numerous following the feeding of lactose. *Atkinson et al.* (1957) reviewed the role of lactose in animal and human nutrition and stated that lactose has a pronounced effect on the intestinal tract of all species. This effect is characterized by a lowering of pH and a subsequent change of intestinal flora to an acidophilic type together with the stimulation of B vitamin synthesis by the intestinal bacteria in both mammals and birds.

Dried skim milk as a source of protein to pigs is generally found to give superior growth and performance as compared with other sources of protein

(*Clausen* 1960a). In order to study this phenomenon, *Sewell & West* (1964, 1965) conducted growth experiments and digestion trials to determine whether the lactose contributed by the skim milk was of importance in protein utilization. The addition of pure ß-lactose to isolated soya bean protein diets at a level corresponding to that provided in the dried skim milk diet produced a significant increase in growth response compared to that obtained with the skim milk diet. *Lassota* (1967) also reported an increase in protein utilization in baby pigs with the addition of lactose. *Thorbek et al.* (1961) obtained an increase in protein deposition in piglets from 30–65 days old when fed casein + lactose compared to casein + glucose and sucrose respectively. A further investigation by *Thorbek & Ludvigsen* (1961) showed no positive effect on protein utilization due to lactose. However, the above authors emphasized that the experimental animals suffered from diarrhoea, particularly at the beginning of the experiment. In experiments with bacon pigs carried out by *Clausen* (1960b) casein was found to be inferior to skim milk powder. A compensation for citric acid and lactose in the skim milk powder had no positive effect on the pigs receiving casein.

Several of the experiments referred to above indicate, however, that lactose may play an important physiological role in protein utilization.

B. Present investigations

In order to study whether the response to lactose is dependent upon the type of protein fed, a number of N balance experiments were carried out with rats. The various protein sources employed were fed with and without a lactose supplement. Lactose was fed in quantities equivalent to a skim milk diet, i.e., approximately 14% of dry matter.

The results of these experiments (Table 41) demonstrate that lactose does not affect the digestibility of the proteins. This is in agreement with the findings of *Chang* (1962) and *Spivey et al.* (1958).

BV, however, is in most cases improved by a lactose supplement. It should be stressed that BV in skim milk powder is considerably higher than in casein, despite the higher content of all essential amino acids in the latter case (*Eggum* 1968a). The addition of methionine to the diet gave rise to a marked increase in BV from 71.3 to 82.1 and a further increase to 87.2 ($P < 0.01$) was obtained with lactose supplement. The proteins from soya bean meal, fish meal and egg (not ether extracted) all had higher BV values ($P < 0.001$) when lactose was added than in diets without lactose. In the rat diet and in barley, however, the addition of lactose had no appreciable effect on biological value. This supports the suggestion that the effect of lactose is dependent upon the type of protein matter concerned. A positive effect would appear to be obtained

Table 41. The influence of lactose on the utilization of different protein sources
Tabel 41. Effekten af laktose på udnyttelsen af forskellige proteinkilder

Protein source	TD (%)	BV (%)	NPU (%)
Skim milk powder	92.8	83.4	77.4
Casein	101.1	71.3	72.1
Casein + 1% methionine	98.3	82.1	80.7
Casein + 1% methionine + lactose	97.1	87.2	84.6
Soya bean meal	90.7	62.0	56.2
Soya bean meal + lactose	89.0	67.9	60.3
Fish meal	92.3	75.1	69.4
Fish meal + lactose	91.8	81.6	74.9
Egg (freeze-dried)	93.9	94.4	88.6
Egg (freeze-dried) + lactose	94.9	98.3	93.3
Rat diet (commercial)	87.6	73.0	63.9
Rat diet + lactose	87.1	72.3	63.0
Barley	80.8	69.9	56.5
Barley + lactose	83.0	69.8	58.0

only when readily digestible proteins are fed. This is in accordance with the theory previously outlined that lactose may retard protein absorption and in this way have a benefical effect on the utilization of readily digestible proteins. Since the protein in the rat diet and in barley has a low digestibility and a lower rate of absorption than the other protein sources fed, the addition of lactose to the rat diet and to barley has no appreciable effect on protein utilization. It is thus tempting to assume that some proteins might be absorbed too rapidly for the organism, i.e., the sites of protein synthesis may not be able to keep up with the absorption velocity and deamination of useful amino acids might take place. Several other workers (*Krehl et al.* 1946, *Harper & Katayama* 1953), however, have reported that when sucrose replaces starch in the diet there is a fall in the nutritive value of the protein. This is explained by the rapid absorption of sucrose compared with the more continuous supply of energy from starch; the latter accompanies the relatively slow and continuous release of the amino acids during digestion.

It would appear from this study that in addition to the effect on protein utilization, lactose may possibly influence several other important factors such as pH in the intestinal tract and hence the microbial flora and intestinal vitamin synthesis. Furthermore, the change in pH will influence mineral and fat absorption; a lower pH in the alimentary tract will result in a higher frequency of mineral and fatty acid ions and consequently facilitate absorption.

It is clear from this discussion that several questions remain concerning the role of lactose in nutrition; certain of these aspects have been taken up for further study.

CHAPTER XII

The influence of heat processing on protein quality

A. General discussion

Prolonged storage, together with several of the procedures employed in the processing of foods and feedstuffs, can have a deleterious or a benefical effect on protein quality. The principal factors involved are the duration of the heat treatment, temperature level and the presence of moisture and reducing substances. The content and availability of several, probably all, amino acids can be affected during processing – see Table 16 (*Ford* 1962, *Eggum* 1969b).

Heat damage to proteins can result from several types of reaction some of which are discussed below. The most severe form of damage is destruction by the transformation of nitrogen to other non-protein constituents. However, one of the most common types of damage reaction is that in which protein is rendered biologically unavailable. Finally the protein may become unpalatable but nevertheless possess a certain nutritive value.

Early work, however, was chiefly devoted to the lysine-sugar reaction (*Maillard* 1912). The different reaction products between carbohydrates and amino acids were first identified by *Maillard* after whom the reaction is named. The *Maillard* reaction can take place at relatively low temperatures, but at a slow rate. According to *Görnhardt* (1955) an increase in temperature of 10°C results in a fourfold increase in the reaction velocity of the *Maillard* reaction. It would thus appear that any benefical effect due to heat treatment might easily be exceeded and result in irreversible damage.

According to *Bender* (1970) the following reactions have so far been demonstrated: »(1) reaction between the amino groups of the amino acids and a reducing substance (in the case of lysine the terminal amino group becomes bound but little is known of the reactions of other amino acids); (2) reaction between the terminal amino group of lysine and the carbonyl secondary decomposition products of autoxidizing fats; (3) protein-protein interaction (carbon-nitrogen links) independent of the presence of reducing substances (*Lea* 1958, *Ellis* 1959).«

It is thus evident that the conditions for deleterious effects on proteins will always be present in the processing, preservation and storage of foods and feedstuffs. This will have certain nutritional and physiological consequences which must be taken into account.

Lysine is regarded as the most heat sensitive amino acid and consequently the preferable seal of damage (*Carpenter et al.* 1962). In measurements with

rats *Block et al.* (1946) found a PER value of 3.5 in an unheated diet. This value was reduced to 2.4 after baking at 200°C for 15–20 min and a further reduction to 0.8 occurred after toasting at 130°C for 40–60 min. The addition of lysine restored the PER to the initial value, but this does not necessarily mean that other non-limiting amino acids were unaffected. Experiments conducted by *Eggum* (1967b, 1967c) showed that baking bread reduced the lysine, tryptophan, methionine and cystine contents of both rye and white bread. Thus BV data obtained with rats on the crust showed a considerable decrease compared to those fed on dough. This is in agreement with the results of *Bender* (1962) who showed that sulphur-containing amino acids might suffer damage from heat, in some cases to an even greater extent than lysine.

Ford (1962) showed by microbiological assay of a variety of materials that the availability of several amino acids was reduced to approximately the same extent after heat treatment. Similar results were obtained by *Eggum* (1969b) in experiments to compare heat-processed and freeze-dried grass meal. *Mauron et al.* (1960) found lysine, methionine and tryptophan to be rendered unavailable when enriched high protein biscuit was subjected to heat treatment. This would appear to suggest that biscuit is possibly not the most suitable vehicle for protein enrichment due to deterioration of protein quality during manufacture.

In investigations carried out with barley, *Eggum et al.* (1969) found that grain drying at 100°C for 30–40 min resulted in a decrease in lysine, TD and BV. *Mason & Weidner* (1964) subjected fishmeal to different treatments and found that autoclaving at 120°C resulted in a drastic decrease in the fluordinitrobenzene-available lysine. An extensive destruction of certain amino acids was recorded, especially cystine and serine, although histidine, threonine, aspartic acid and lysine also suffered considerable damage. Similar results were obtained in investigations by *Madsen et al.* (1965); the autoclaved fish meal was inferior to unheated meals when fed to bacon pigs. *Smith & Scott* (1965) reported similar results and also found a decrease in threonine availability after treatment of fish meal. *Homb* (1962), however, found no significant differences in N retention in pigs fed fish meal subjected to different heat treatments.

Among the positive effects of heat treatment protein denaturation by moderate heat can be mentioned. This facilitates the enzymatic decomposition of the proteins and thus improves digestibility (*Gitler* 1964). Heat treatment can also result in the decomposition of hydrogen bonds, salt bonds and peptide linkages, thereby increasing the number of reactive groups in the protein molecule. Furthermore, heat can inactivate enzyme inhibitors and neutralize toxic substances (*Lang* 1960). *Bender* (1970) reports that the nutritive value of most, if not all, legumes is improved by heating. This is apparently partly due to the destruction of trypsin inhibitors and toxic substances and partly

to improved digestibility and availability of the sulphur-containing amino acids. Extensive studies of soya bean protein have established that a product of the highest nutritive value is obtained only if heat application establishes equilibrium between benefical and deleterious effects.

Similar beneficial effects of heat have been reported in cereals. *Moran et al.* (1968) found in experiments with raw wheat germ that considerable quantities of antitrypsin could be destroyed by autoclaving for 20 min. *Laporte & Tremoliéres* (1962) reported that wheat, rye and buckwheat, and to a lesser extent rice, oats and maize, contain a trypsin inhibitor. This factor could be destroyed in rice, wheat and oats by boiling but not in the other cereals mentioned.

The improvement of taste by the formation of various aromatic substances is a further beneficial effect brought about by heat treatment (*Hertz* 1960). This factor is of particular importance in human nutrition, but will not be discussed here.

Several factors would appear to influence protein damage, some of the most important of which will be discussed in the following.

1. Reducing substances

One of the major causes of reduction in nutritive value appears to be the *Maillard reaction* (1912) between lysine and a reducing substance (*Carpenter & Ellinger* 1960, *Dammers* 1964, *Erbersdobler & Zucker* 1966, *Bujard et al.* 1967). The free ε-amino group of the lysine molecule might form enzyme-resistant bonds with reducing substances and consequently become unavailable to the organism. Such bonds are generally broken by the prevalent procedures of hydrolyses (*Friedman & Kline* 1950a, *Friedman & Kline* 1950b). This is the main reason why the absolute lysine content in heat-damaged proteins cannot give an exact estimate of the available lysine. It should be emphasized that amino acids liberated by acid hydrolysis do not generally provide any precise information regarding the availability of the single amino acid to the living organism. Only direct measurements of the availability provide the required information.

There are numerous reports of the stability of proteins to heat until a reducing substance is introduced into the system. *Erbersdobler* (1966) found that autoclaving muscle protein alone had no effect on protein quality, whereas the addition of glucose resulted in a marked decrease in available lysine and PER after autoclaving for 20 min. Similar results were obtained by *Halevy & Guggenheim* (1953) with gluten. The biological value of gluten was reduced from 55 to 18 when heated with glucose. *Clegg* (1960) found that supplements of skim milk to biscuit reduced available lysine by 50%, while casein caused no reduction. This condition is due to the lactose in skim milk and also indicates that the intact starch present in the biscuit did not have the same delete-

rious effect on available lysine as lactose. *Hawthorne & Brooks* (1944) and *Tarr* (1954) found that even small amounts of reducing substances are capable of causing damage as shown with egg and fish.

2. Moisture

Carpenter et al. (1962) showed that the loss of available lysine was greatest at 5–14% moisture. This would suggest that drying to a water content below 14% should only take place when absolute necessary. This aspect should be taken into account when drying grain and especially in grass meal production as discussed by *Eggum* (1969b). Grass is particularly rich in reducing substances, free amino acids and proteins with high lysine density. When this substrate is processed at a moisture content of 10 to 8% it is obvious that the *Maillard* reaction has the most favourable conditions.

Miller (1956), however, reported that dry materials are resistant to heat, while *Eggum* (1968a) found that boiling meat in excess water usually had no effect. *Eggum* (1969c) compared the sterilization effect on dry (11.2% H_2O) and soaked (71.6% H_2O) rat diets. In both cases a severe reduction in lysine content and NPU was found, although the effect was not nearly as pronounced on the soaked material as on the dry diets. Sterilization by irradiation was the mildest method with regard to protein quality.

In the following, however, only a few examples of processing used under practical conditions will be discussed.

B. Present investigations

1. The effect of boiling on protein quality in grain

Interest in the use of feed of vegetable origin for fur-bearing animals has improved over the last years. Experiences, however, have shown that the animals appear to do better if such feedstuffs undergo some processing (*Eggum & Jørgensen* 1971). Grain proteins are generally boiled or steamed before use and the question arises as to whether such treatments affect the protein quality. In order to demonstrate possible effects grains of barley, oats, rye, maize and sorghum were ground, mixed with water (1:3) and the resulting mixture boiled for 30 min under constant stirring. After processing the samples were immediately freeze-dried and prepared for analysis. The treated samples were compared with similar untreated samples with regard to trypsin inhibitor (urease activity), AAC, TD, BV and NPU. The results obtained with barley, oats and rye are shown in Table 42 and those from rye, maize and sorghum in Table 43.

The urease activity was found to be extremely low in both boiled and control samples of barley, oats and wheat. In comparison a urease activity of 0.18 meq/g was recorded in soya bean meal of excellent quality, thereby suggesting an extremely low trypsin inhibitor concentration in barley, oats and wheat.

Table 42. The influence of boiling on protein quality in barley, oats and wheat
Tabel 42. Kogningens indflydelse på proteinkvaliteten i byg, havre og hvede

Sample	Barley		Oats		Wheat	
Boiled (min)	0	30	0	30	0	30
Urease activity (meq/g)	0.04	0.03	0.04	0.04	0.06	0.04
	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)
Lysine	3.36	3.25	4.25	3.97	2.98	2.88
Methionine	1.88	1.45	2.00	1.75	1.88	1.70
Cystine	2.10	1.90	2.59	2.29	2.49	2.10
Aspartic acid	6.18	5.56	8.12	8.01	5.24	5.19
Threonine	3.16	3.17	3.41	3.38	2.99	2.88
Serine	4.16	4.01	4.52	4.45	4.57	4.22
Glutamic acid	25.19	26.69	21.54	21.24	32.36	29.73
Proline	10.86	10.92	4.96	4.72	9.55	8.60
Glycine	4.02	3.65	4.80	4.71	4.02	3.85
Alanine	4.11	3.74	4.61	4.49	3.79	3.61
Valine	4.91	4.97	5.39	5.00	4.69	4.39
Isoleucine	3.56	3.54	3.82	3.68	3.63	3.54
Leucine	7.01	6.85	7.34	7.21	7.08	6.76
Tyrosine	3.21	3.33	3.35	3.63	3.04	2.56
Phenylalanine	5.00	5.14	5.24	4.98	4.56	4.47
Histidine	2.06	1.99	2.32	2.16	2.32	2.21
Arginine	5.08	4.66	6.49	6.47	4.86	4.75
Tryptophan	1.28	1.05	1.21	1.17	1.36	0.98
Protein value expressed as a percentage:						
TD	84.6	84.8	89.9	94.4	90.7	93.2
BV	77.5	72.4	77.5	79.1	68.2	66.5
NPU	65.3	61.4	69.7	74.4	61.8	63.7

Amino acid analyses showed certain essential amino acids, chiefly lysine, methionine and tryptophan, to decrease when boiled for 30 min. The treatment resulted in a significant reduction in BV in barley ($P < 0.001$), whereas no significant differences in BV were found in oats and wheat. In these latter species, however, a beneficial effect of heat on TD was found, although significant ($P < 0.01$) only in the case of oats.

Table 43. The influence of boiling on protein quality in rye, maize and sorghum
Tabel 43. Kogningens indflydelse på proteinkvaliteten i rug, majs og milokorn

Sample	Rye		Maize		Sorghum	
Boiled (min)	0	30	0	30	0	30
Urease activity (meq/g)	0.06	0.04	0.07	0.04	0.09	0.05
	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)
Lysine	3.96	4.00	2.96	2.69	1.81	1.73
Methionine	1.79	1.70	2.26	2.17	1.73	1.68
Cystine	2.00	1.97	2.05	1.76	1.28	1.21
Aspartic acid	7.61	7.52	6.39	6.38	7.68	7.93
Threonine	3.42	3.36	3.64	3.63	3.48	3.49
Serine	4.11	4.18	4.72	4.78	4.73	4.81
Glutamic acid	23.58	23.65	20.67	21.98	22.00	22.06
Proline	8.51	8.16	8.99	9.19	8.21	8.41
Glycine	4.40	4.30	3.72	3.74	3.11	3.18
Alanine	4.34	4.35	7.27	7.29	9.56	9.48
Valine	4.67	4.77	4.89	4.82	5.38	5.46
Isoleucine	3.53	3.43	3.56	3.39	4.36	4.29
Leucine	6.34	6.37	12.61	12.75	11.83	11.64
Tyrosine	2.73	2.91	4.42	4.28	4.03	4.16
Phenylalanine	4.33	4.46	5.01	4.89	5.31	5.19
Histidine	3.03	2.27	2.97	2.88	2.05	2.10
Arginine	5.26	5.35	4.54	4.66	3.47	3.39
Tryptophan	1.51	1.14	0.87	0.71	0.94	0.87
Protein value expressed as a percentage:						
TD	79.4	78.3	95.7	93.8	87.3	88.1
BV	74.3	78.7	62.4	61.4	48.4	48.1
NPU	58.9	61.6	59.7	57.6	42.5	42.4

An extremely low urease activity was also found in the case of rye, maize and sorghum (Table 43). This is contrary to the results of *Moran et al.* (1968) and *Laporte & Tremolières* (1962) who found considerable amounts of antitrypsin and consequently obtained a benefical effect from heat treatment.

The reduction in tryptophan content by processing was probably due to the acid labile nature of this amino acid; the pH of the barley suspension was 5.4. This might also explain the decrease in the content of sulphur-containing amino acids (*Eggum* 1970c).

No significant change in the true digestibility of the samples was found after heat treatment, whereas BV in rye showed a marked increase. This increase is barely significant due to the relatively large variation in the BV figures. The increase in BV in rye, however, might be the result of heat decomposition of ergot or a substance of phenol character as described by *Wieringa* (1967).

In general the data listed in Table 42 and 43 suggest a positive effect on TD in oat protein and BV of rye due to boiling. No positive effects were observed with regard to the other factors investigated, but indications of deleterious effects were found in the case of BV in barley and in lysine, tryptophan, methionine and cystine contents in almost all of the samples evaluated.

The lack of agreement concerning the effect of heat treatment can possibly be due to the technique employed. Using the PER method *Shyamala & Kennedy* (1962) demonstrated that the value of whole wheat flour increased from 1.65 to 2.04 on heating the dough to form chappatis. *Bender* (1970) considered this effect to be due to the greater palatability of the heated diet and it would thus appear that reports of improved nutritive value due to heat treatment also represent a criticism of the method of assay employed. This is often the case when growth is involved.

2. The influence of autoclaving of fish protein under different conditions

In order to further illustrate the problems discussed above experiments were carried out to evaluate the protein quality of fish forcemeat subjected to different processing treatments. The samples were prepared by Lars Herborg, Food Technology Laboratory, Technical University, Copenhagen and kindly placed at the disposal of the author. The results are shown in Table 44. The samples were derived from cod of excellent quality and the crude forcemeat contained 83.7% water. With the exception of treatment B, all samples were freeze-dried after processing. Sample D was subjected to heat treatment alone and sample E was supplemented with 5% potato starch.

As can be seen from Table 44, processing had no appreciable effect on amino acid content, even in the case of the heat-sensitive lysine. This is probably due to the high water content in the material employed and the resulting reduction in the velocity of the *Maillard* reaction.

Although the true digestibility was hardly affected, the biological value clearly indicates both benefical and deleterious effects of heat treatment. BV was significantly lower ($P < 0.001$) in the freeze-dried sample than after roller-drying, due perhaps to destruction of thiaminases during processing. Results with sample E demonstrate that the addition of starch to the forcemeat before processing had a negative influence on BV; this value showed a significant reduction ($P < 0.001$) from 85.7 in sample D to 71.3 in sample E which were subjected to the same processing treatments. The extremely severe reduction in protein quality which occurs when protein is processed together with reducing substances is a problem which warrants considerably more attention. The danger is thus far more serious when complete diets are processed; this is particularly the case with baby food where the various ingredients

Table 44. The protein quality in forcemeat of fish after different treatments
Tabel 44. Proteinkvaliteten i fiskefars efter forskellig behandling

Sample	A	B	C	D	E	F	G
Treatment	Freeze-dried	Roller-dried	Heat 30 min 110°C	Heat 25 min 117°C	As D + 5% starch	Heat 13 min 119°C	Heat 40 min 118°C
	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)
Lysine	8.94	8.61	8.44	8.88	8.72	8.53	8.99
Methionine	3.07	3.05	2.98	2.98	2.61	3.11	2.80
Cystine	0.93	1.02	1.00	0.92	0.95	1.05	0.96
Aspartic acid	9.70	9.23	9.19	9.86	10.06	9.30	9.15
Threonine	4.08	3.93	3.88	4.04	4.07	3.94	3.99
Serine	3.92	3.77	3.82	3.90	3.90	3.76	3.74
Glutamic acid	15.86	14.89	14.92	15.39	15.41	14.98	14.50
Proline	3.29	3.03	3.04	2.97	3.21	2.97	2.88
Glycine	4.44	4.45	4.19	4.49	4.28	4.26	3.64
Alanine	5.62	5.37	5.28	5.59	5.61	5.40	5.07
Valine	4.68	4.61	4.72	4.92	4.99	4.84	4.59
Isoleucine	4.34	4.46	4.25	4.61	4.46	4.26	3.95
Leucine	7.65	7.47	7.27	7.72	7.77	7.50	7.00
Tyrosine	3.42	3.40	3.09	3.12	3.36	2.98	2.95
Phenylalanine	3.74	3.61	3.61	3.73	3.84	3.97	3.63
Histidine	2.37	2.13	2.09	2.23	2.12	1.96	2.55
Arginine	6.06	5.81	5.61	5.99	5.89	5.73	6.32
Tryptophan	1.18	1.34	1.15	1.30	1.14	1.10	1.09
Ammonia	1.11	1.10	1.05	1.13	0.82	1.08	1.17
Protein value expressed as a percentage:							
TD	96.4	97.8	96.2	95.2	95.4	96.3	94.3
BV	77.7	84.6	88.2	85.7	71.3	83.7	82.2
NPU	74.9	82.9	84.9	81.5	68.0	80.6	78.1

are mixed prior to processing. Two samples of baby food evaluated by *Eggum* (1968a) were of extremely poor protein quality and would fail to supply babies with adequate amounts of available amino acids.

The extent of the reduction in protein quality is considerably lower when proteins with few reducing substances are processed (Table 44). Processing forcemeat alone for 40 min at 118°C has only a slight deleterious effect on the protein quality.

3. The influence of pelleting on protein quality in pig diets

Homb et al. (1964) and *Antoni & Cranz* (1967) compared meal and pelleted diets fed to pigs but found no significant differences in quality. Similarly, in experiments with horses pelleting was not found to have any effect on

rate of gain or feed efficiency ratio. *Schulz* (1967), however, showed that protein in pellets had a higher digestibility in pigs than protein in meal, whereas *Meade et al.* (1966) found an increase in feed utilization after pelleting. In extensive investigations carried out by *Hansen* (1970) with growing pigs comparisons of meal and pellets were made. No significant differences were recorded in feed utilization or carcass quality, although pigs fed pellets suffered more from diarrhoea.

In an attempt to determine the effect of the pelleting process on protein quality, 3 diets of meal and pellets prepared from identical materials were evaluated. All diets were obtained from the Progeny Testing Section of the Department for Experiments with Pigs and Horses, National Research Institute of Animal Science. The three sets of diets were collected in 1970 at different times, i.e., they were not manufactured simultaneously. The results of the amino acid analyses and protein value when fed to rats are shown in Table 45.

Table 45. The influence of pelleting on protein quality of pig diets
Tabel 45. Pelleteringens indflydelse på proteinkvaliteten i svinefoder

Sample	Meal 1	Pellets 1	Meal 2	Pellets 2	Meal 3	Pellets 3
	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)	(g/16gN)
Lysine	4.90	4.59	5.04	4.72	5.27	4.93
Methionine	1.53	1.50	1.38	1.47	1.54	1.48
Cystine	1.66	1.35	1.66	1.53	1.44	1.46
Aspartic acid	7.69	7.34	8.22	8.08	8.40	8.62
Threonine	3.46	3.31	3.59	3.63	3.65	3.67
Serine	4.15	4.05	4.51	4.41	4.49	4.50
Glutamic acid	21.15	20.90	21.63	21.58	21.24	21.82
Proline	8.31	8.26	8.16	8.23	8.31	8.29
Glycine	4.65	4.55	6.00	4.79	5.96	5.22
Alanine	4.30	4.17	4.88	4.42	4.49	4.51
Valine	4.95	4.60	5.07	5.03	5.08	5.39
Isoleucine	3.88	3.67	3.79	3.89	3.99	4.15
Leucine	6.98	6.78	7.22	7.22	7.59	7.74
Tyrosine	3.24	3.67	3.61	3.62	3.56	3.53
Phenylalanine	4.76	4.81	4.74	4.64	4.97	4.85
Histidine	2.32	2.20	2.22	2.24	2.21	2.36
Arginine	5.93	5.86	6.10	5.84	5.86	5.93
Tryptophan	1.36	1.38	1.21	1.20	1.37	1.28
Protein value expressed as a percentage:						
TD	86.5	86.8	85.2	84.1	85.8	84.3
BV	70.7	69.8	71.8	68.0	77.2	75.1
NPU	61.2	60.6	61.2	57.2	66.2	63.3

The amino acid analyses showed the lysine content to be 6–8% lower in pellets than in meals whereas no significant differences were found in the case of the other amino acids. This reduction in total lysine did not lead to a significant decrease in BV; the NPU value was found to be lower in pellets than in meal, although the difference was found to be significant only in the latter samples ($P < 0.05$). This absence of any significant effect on BV by the reduction in lysine is probably due to the fact that another amino acid was the limiting factor. This again demonstrates the fact that changes are induced in the biological parameters only when a change occurs in the limiting amino acid.

It can therefore be concluded on the basis of the data in Table 45 that the pelleting process can influence the total and available contents of amino acids and consequently the protein utilization.

From this discussion regarding the influence of heat treatment on protein quality it must be evident that conditions for deleterious influence on protein will always be present during processing, preservation and storage of foods and feedstuffs. The nutritional and physiological consequences involved warrant much more detailed consideration than has been given in the past and hence a better propagation of knowledge concerning these aspects is required.

CHAPTER XIII

The value of cystine as a substitute for methionine

A. General discussion

Using nitrogen retention as a criterion, *Mitchell et al.* (1968) found that cystine could supply at least 70% of the sulphur-containing amino acid requirement of weaning pigs. This value is considerably higher than earlier estimates of 40% by *Becker et al.* (1955) and 50% by *Shelton et al.* (1951) in which weight gain was used as the criterion. It is more in line with the 68% replacement value reported for the growing rat by *Rao et al.* (1961). *Baker et al.* (1969) showed in experiments with growing pigs that weight gain was as rapid when 56% of the total sulphur-containing amino acids were provided by cystine as when all were supplied by methionine. In nitrogen retention assay they indicated that cystine could supply at least 66% of the requirement without affecting nitrogen retention. They concluded that maximal performance could be achieved with a diet containing 66% of the total sulphur amino acids as cystine.

It is conceivable that the disparities noted in the magnitude of cystine substitution effect may have resulted from differences in assay methodology. The replacement value will also depend on methylating agents such as choline, betaine and vitamin B_{12} present in the diet. In addition, the lack of information concerning the order of priority of protein synthesis and transmethylating reactions under conditions of methionine deficiency renders the evaluation of cystine as a substitute for methionine extremely difficult.

In order to elucidate certain aspects of cystine substitutes nitrogen retention assays were carried out with rats. Control diets of casein and soya bean meal were employed since these proteins are known to be limited by the sulphur-bearing amino acids.

B. The value of cystine as a substitute for methionine as determined in experiments with soya bean meal and casein

Soya bean meal is low in both cystine and methionine, while casein has a high methionine but an extremely low cystine content. These differences in the original contents of the sulphur-bearing amino acids proved useful in estimating the effect of adding cystine or methionine to these sources.

1. Soya bean meal as protein source

Table 46 shows the influence on TD, BV and NPU of adding cystine or methionine to soya bean meal.

Table 46. The effect on TD, BV and NPU of the addition of cystine or methionine to soya bean meal

Tabel 46. Indflydelsen på SF, BV og NPU ved tilsætning af cystin eller methionin til sojaskrå

	Content of sulphur-bearing amino acids in the diets						
	Cystine (g/16gN)	Methionine (g/16gN)	Cystine + methionine (g/16gN)	Cystine in % of cystine + methionine	TD (%)	BV (%)	NPU (%)
Soya bean meal	1.56	1.61	3.17	49.2	86.0	64.3	55.3
» + cystine	1.96	1.61	3.57	54.9	86.3	69.3	59.9
» + methionine	1.56	2.01	3.57	43.7	86.1	70.2	60.3
» + cystine	2.20	1.61	3.81	57.7	87.7	80.2	70.3
» + methionine	1.56	2.25	3.81	40.9	86.4	85.7	74.0
» + cystine	2.60	1.61	4.21	61.8	86.6	80.5	69.7
» + methionine	1.56	2.65	4.21	37.1	86.1	88.6	76.3
» + cystine	3.00	1.61	4.61	65.1	85.2	80.9	68.9
» + methionine	1.56	3.05	4.61	33.8	87.4	85.7	74.9

It appears from Table 46 that supplements of cystine as well as methionine to soya bean meal had a beneficial effect on biological value while true digestibility was unaffected. At the lowest supplements the influence of cystine or methionine on BV was exactly the same. At this level cystine constituted 54.9% of the content of cystine + methionine. The highest BV (88.6), however, was obtained when methionine was supplemented. Cystine accounted for only 37.1% of the sulphur-bearing amino acids at this level. These results indicate that cystine appears to have higher replacement values for methionine at the lower BV values. For further illustration of this question other experiments with soya bean meal were carried out where both cystine and methionine were added simultaneously. The results are seen in Table 47.

A simultaneous addition of both cystine and methionine shows that the highest BV can be obtained even if cystine constitutes 50% of the total content of sulphur-bearing amino acids. Furthermore Table 47 shows that the adjusted ratios between cystine and methionine did not affect the BV severely as almost all BV values were in the range 85–90%. However, the practical rations employed for our farm animals very seldomly contain more cystine than methi-

onine. This justifies the expression of the requirements as cystine + methionine. This is at least more correct than giving a requirement for methionine regardless of the cystine content.

Table 47. The effect on TD, BV and NPU of the addition of cystine and methionine to soya bean meal
Tabel 47. Indflydelsen på SF, BV og NPU ved tilsætning af cystin og methionin til sojaskrå

	Content of sulphur-bearing amino acids in the diets						
	Cystine (g/16gN)	Methionine (g/16gN)	Cystine + methionine (g/16gN)	Cystine in % of cystine + methionine	TD (%)	BV (%)	NPU (%)
Soya bean meal	1.56	1.61	3.17	49.2	86.0	64.3	55.3
» + cyst. + meth.	1.88	1.88	3.76	50.0	86.7	84.3	73.1
» + »	2.20	1.88	4.08	53.9	86.1	84.2	72.4
» + »	2.60	1.88	4.48	58.0	87.7	85.0	74.6
» + »	1.88	2.20	4.08	46.1	87.6	88.9	77.9
» + »	1.88	2.60	4.48	42.0	86.3	87.9	75.8
» + »	1.88	3.00	4.88	38.5	86.3	85.8	74.1
» + »	2.20	2.20	4.40	50.0	87.8	89.4	78.5
» + »	2.60	2.20	4.80	54.2	87.4	87.0	76.0
» + »	3.00	2.20	5.20	57.7	87.0	87.6	76.2
» + »	2.60	2.40	5.00	52.0	87.4	87.9	76.8
» + »	2.60	2.80	5.40	48.1	86.8	90.4	78.5
» + »	2.60	3.20	5.80	44.8	87.3	88.7	77.4

2. Casein as protein source

As mentioned above the cystine content in casein is very low. This provides a possibility of examining whether a specific requirement exists for cystine above that already present in casein by the addition of cystine or methionine. The results from the experiments with casein are shown in Table 48.

As seen in Table 48 supplements of either cystine or methionine produced similar improvements. This is probably due to differences in the original contents. Casein has a very low cystine content but a high methionine content and thus a supplement of cystine will have the same effect on BV as methionine. With soya bean meal, however, the situation was somewhat different.

The results in Table 48 suggest that rats have a very low specific requirement of cystine provided sufficient methionine is available. This is in agreement with the work reported with pigs by *Baker et al.* (1969).

Table 48. The effect on TD, BV and NPU of the addition of cystine or methionine to casein

Tabel 48. Indflydelsen på SF, BV og NPU ved tilsætning af cystin eller methionin til kasein

	Content of sulphur-bearing amino acids in the diets						
	Cystine (g/16gN)	Methionine (g/16gN)	Cystine + methionine (g/16gN)	Cystine in % of cystine + methionine	TD (%)	BV (%)	NPU (%)
Casein	0.58	3.09	3.67	15.8	99.4	71.3	70.9
» + cystine	0.98	3.09	4.07	24.1	99.6	76.2	75.9
» + »	1.38	3.09	4.47	30.9	99.4	82.3	81.8
» + »	1.80	3.09	4.89	36.8	99.5	86.1	85.5
» + »	2.25	3.09	5.34	42.1	99.7	85.9	85.6
Casein + methionine	0.58	3.89	4.47	13.0	99.6	82.8	82.5
» + »	0.58	4.30	4.88	11.9	99.0	86.6	85.6
» + »	0.58	4.70	5.28	11.0	99.3	86.2	85.6

Owing to the experimental technique used in present experiments no statement can be made of the maximal value of cystine as a substitute for methionine. The only conclusions possible are that cystine can constitute at least 50% of the total content of sulphur-bearing amino acids and that the specific requirement of cystine appears to be very low. This information must be of value as practical rations employed for farm animals very seldomly contain more cystine than methionine. Consequently cystine can be considered as biological efficient as methionine under practical conditions. The low specific requirement of cystine gives rise to the possibility of enriching diets low in sulphur-containing amino acids with low cost methionine.

CHAPTER XIV

General discussion

As implied by the title, the present work is intended as a study of certain factors which might affect protein utilization in rats and pigs. The protein value is expressed as:

1. Amino acid composition (AAC)
2. Available amino acids (AAA)
3. True digestibility of total protein (TD)
4. Biological value (BV)
5. Net protein utilization (NPU)
6. Utilizable nitrogen (UN)

Thus the method of *Mitchell* (1924a) forms the basis of the majority of the biological results obtained. However, this method has been criticized for several reasons. Thus the pattern of amino acids requirement for growth may differ from that required for maintenance and the optimum patterns may also vary with the physiological state of the individual. *Mitchell & Beadles* (1950) demonstrated that relatively small amounts of lysine are required for maintenance in the rat compared with the amount needed for growth. *Fisher et al.* (1960) stated that the differential response to two protein levels can be explained in terms of the relatively greater methionine requirement for maintenance at the low protein level compared to the relatively higher lysine to methionine ratio required for rapid growth at the high protein level. In general, however, data suggest that the general pattern optimum for growth is also the most suitable for maintenance and the repletion of depleted tissues (*Mitchell* 1959).

In discussing the amino acid requirement for maintenance contra tissue synthesis, a high lysine content of metabolic protein (Table 9) does not appear to suggest a low lysine requirement for maintenance. Furthermore endogenous nitrogen must originate from protein high in lysine which also contradicts a hypothesis of low lysine requirement for maintenance. A high methionine requirement for maintenance is quite obvious since methionine is the principal methyl donor in the body. The amino acid composition of metabolic protein of rats and pigs is very much the same, suggesting similarities in protein metabolism.

Metabolic protein is found to be positively correlated with dry matter consumption. In experiments with pigs both nitrogen digestion and nitrogen retention were found to be independent of age in the interval 30–60 days. This

was true for both vegetable and animal protein. Consequently the results obtained at any age-interval between 30 and 60 days could be compared directly. This direct comparison was also shown to be valid for the availability of the individual amino acids. In calculating amino acid availability the method of *Kuiken & Lyman* (1948) was employed. This method, however, has been criticized on the basis of the microbial activity in the intestinal tract. A study of this problem did not indicate any serious disturbances due to the microflora in the experimental diets employed. Diets supplemented with bactericid material were compared with unsupplemented diets but no real differences in the availability of amino acids were found. An exchange of carbohydrate source was also found to have no significant effect. In addition stimulating reports of other workers on the same topic encouraged the author to employ the faecal analysis method in calculating the true digestibility of individual amino acids.

Fifteen different protein sources were evaluated in both rats and pigs with regard to the criteria listed above. It appears from this work that the TD of several amino acids differs significantly from the TD of total N. This was particularly marked in several grain proteins which showed low values for lysine, aspartic acid, glycine and alanine, whereas glutamic acid, histidine and arginine were generally found to possess higher TD values than total N. This is in agreement with the results of other workers obtained from both in vivo and in vitro experiments. The low value for lysine is especially unfortunate, as this amino acid is the limiting factor in many proteins. In maize and concentrated feedstuffs the availability of the amino acids is generally closer to the TD of total N in corresponding material than is the case with most of grain products. The correlation between TD values obtained with rats and pigs respectively was found to be extremely high for both total N and the individual amino acids, tending to enforce the reliability of the method of *Kuiken & Lyman* (1948).

Of the 15 diets fed to both rats and pigs only 6 were fed at the same protein level and are thus directly comparable with regard to BV. However, in these diets higher BV values were obtained in pigs compared to rats, although the correlation coefficient between BV in rats and pigs respectively was 0.86. This relatively high value indicates that these two animal species react similarly to the protein source in spite of differences in the levels of the BV. On the basis of these results it is tempting to conclude that pigs have a lower lysine requirement compared to rats, lysine being the limiting amino acid in grain protein. This is not, however, in agreement with the *NRC* (1968) standards for lysine requirement for rats and pigs respectively. It appears from these standards that pigs possess 1% higher lysine requirement than rats, indicating the higher BV values obtained on pigs to be erroneous. According to *Schiller & Ocio* (1963) the disagreements between BV

values obtained on rats and pigs are due to the higher methionine requirement of rats. As the content of sulphur-containing amino acids in grain protein is relatively high, the disagreement is unlikely to be due to these amino acids. The BV values obtained with pigs for the grain diets are abnormally high, particularly if available lysine is taken into consideration. A possible explanation might be that the experimental periods used for pigs were too short and the lysine pool (*Young* 1970) can thus have influenced the results, giving rise to high BV values. Despite the different levels of the BV of rats and pigs the relative values can nevertheless provide valuable information.

Since the diets 7–15 were fed at different N concentrations to the two animal species, a direct comparison of the BV of these diets is not possible. This is illustrated by feeding increasing amounts of protein from casein and soya bean meal respectively to rats. The BV of both casein and soya bean meal is negatively correlated to the protein concentration in the diet ($r = -0.99$). The regression coefficients differ for the two protein sources, –1.87 for casein but only –1.37 for soya bean meal. This shows that a regression equation obtained for one protein source cannot be used to correct the BV of another source due to differences in the N levels of the experimental diets. The true digestibility, however, is independent of the protein level in the diet, thereby indicating that metabolic N is independent of the protein concentration in the diet. It can also be assumed that the replacement of casein and soya bean meal by autoclaved potato starch does not affect the TD values of total N, an assumption which is also valid for the individual amino acids. The close agreement between TD values of proteins fed at different levels to rats and pigs respectively also support this assumption.

The apparent protein digestibility is readily influenced by the protein content of the diet. AD increases curvilinearly with the N content. This is almost certainly due to the fact that metabolic N accounts for a relatively smaller part of the faecal N with increasing protein in the diet. Consequently values for AD are only valid under strictly standardized conditions, at least with regard to the N concentration in the diet. The disagreement between AD values obtained at different laboratories might well be due to differences in the levels in the experimental diets.

It has been suggested by several workers that a high energy content in the diet might have a protein-saving effect. This could not be confirmed in the present work; a replacement of 14% N-free diet with fat was found to have no effect on either TD or BV. Furthermore no protein-saving effect of fat was observed in experiments with protein of high or low BV respectively. Similar results were obtained when increasing amounts of cellulose powder were included in the diets; the addition of up to 25–30% cellulose powder (low in digestible energy) in the diet had no effect on either TD or BV, i.e., a low content of digestible energy does not affect BV. This applies

only up to a certain limit, however, and the results in Table 40 indicate that when 30% cellulose powder is included in the diet the body may begin to use protein as an energy source. Since TD was found to be unaffected by the cellulose content in the diet, the present experiments do not support the assumption that crude fibre has a negative effect on protein digestibility. Feedstuffs high in crude fibre possess protein of lower availability than feedstuffs with lower crude fibre contents, but this does not imply that crude fibre has a negative effect on protein digestibility.

Several workers have shown lactose to influence protein utilization, although opinions differ as to the reasons for this effect. The present investigations, however, suggest that a positive effect from a lactose supplement is obtained only with easily dissolved proteins, whereas no effect is obtained with more slowly digestible proteins. Other workers have shown that lactose can retard absorption velocity and that this seems to be beneficial for protein utilization in certain cases. It is thus assumed that absorption of amino acids might be too rapid to secure maximum protein utilization.

Several foods and feedstuffs are exposed to certain types of heat processing before consumption. In view of the fact that proteins are heat sensitive, processing might easily have a deleterious effect on protein quality. It is obvious that the effect is correlated with the duration of processing as well as with temperature level. Furthermore the water content and the content of reducing substances in the samples have considerable influence on protein quality of the product. A surplus of water should have a protective effect, whereas the presence of reducing substances can easily have a marked deleterious effect. Hence boiling in surplus of water should not be very harmful. In a study of boiled grain the biological criteria were not found to be influenced to any great extent. However, acid labile amino acids such as tryptophan, methionine and cystine were often destroyed. This would affect protein utilization if one of these amino acids were the limiting factor. Autoclaving can have an extremely deleterious effect on protein quality, particularly if reducing substances are present. This was clearly demonstrated in experiments with forcemeat of fish subjected to different treatments. The BV in autoclaved forcemeat supplemented with 5% starch was considerably reduced (from 85.7 to 71.3) due to the reducing effect of the starch. Pelleting takes place at a low water content in the material (below 14%) and thus the likelihood of deleterious effects is considerably increased. However, the resultant effect appears to be quite moderate, probably due to the relatively short duration of exposure to heat.

A problem often discussed is the value of cystine as a substitute for methionine. The present investigations show that cystine can constitute at least 50% of the total content of sulphur-bearing amino acids and that the specific requirement of cystine is low.

It is pointed out in the present work that the biological value of a protein does not provide a great deal of information on the supplementary effect of the protein, although a high BV will also indicate a high supplementary effect. Diets are generally made up of two or more protein components and thus the BV of the individual feedstuffs would appear to be of limited interest and a complete amino acid analysis of much greater value. However, a choice between these two methods of analysis is irrelevant in that they represent two completely different concepts. The amino acid analysis was considered to be the most suitable chemical analysis in protein evaluation to explain the results obtained in the present work. The results given in Table 44 from experiments with and without starch supplementation to fish forcemeat before autoclaving clearly illustrate this. The BV was found to be drastically reduced in spite of the fact that the amino acid composition was unchanged. Consequently an amino acid analysis alone would fail to demonstrate the real reduction in nutritional quality. It should be emphasized again that the biological value is based on nitrogen balance experiments and the nitrogen utilization given as a percentage. A criticism of the biological value as a criterion of protein quality must then also apply to nitrogen balance experiments.

However, information obtained during recent years have proved the superiority of the *Thomas-Mitchell* equation. It is therefore hoped that the present experiments carried out according to *Thomas-Mitchell* will also add some knowledge to protein metabolism.

CHAPTER XV

Conclusion

True digestibility, biological value and net protein utilization enable a ranking of protein sources on an assumedly absolute scale. However, the necessity is stressed of strictly standardized conditions when the method of *Mitchell* is employed in protein evaluation. In spite of standardized conditions the value assigned to a particular protein source may differ between theoretically equivalent scales.

It would appear from the present experiments that the amounts of the individual amino acids present in the diet are not necessarily the amounts available to the body. When data for amino acid availability have been obtained, the problem remains as to how these are to be used to correct total amino acid data. Clearly the units in which nutrient content of feed are expressed must be the same as those used for the requirement standards. The nutrient requirements have been determined to a large extent by measuring the response obtained when supplementary amino acids are added to a basal diet of natural feedstuffs. The requirement is calculated as the total amino acids supplied by the basal diet plus the amount contributed by that supplement which gives maximum response. As most of this research has been carried out with maize and soya bean meal as a basal diet, *Combs & Nott* (1967) proposed that amino acid data should be corrected by an availability arbitrarily set at 100% for maize and soya bean meal.In principle this appears to be a sensible interim measure.

On a long-term basis, however, the availability of amino acids in all feeds should be determined and the amino acid requirements calculated in terms of available amino acids. The actual amino acid availability could then be used to replace the relative availability factors in drawing up tables of feed composition.

The present experiments show that the nitrogen concentration in the diet does not affect metabolic nitrogen excretion. Consequently the true digestibility of protein and amino acids is independent of the protein in the diet. Apparent protein digestibility and biological value, however, are dependent on the protein concentration in the diet and must therefore be estimated at a fixed level of nitrogen concentration.

Extra energy from fat in the diet does not appear to have any protein-saving effect irrespective of the protein quality of the diet. Furthermore TD and BV are independent of crude fibre content, although feedstuffs high in crude fibre show a low protein digestibility.

Lactose is found to have a positive effect on the utilization of certain proteins. The main effect of a lactose supplement in a diet is suggested to be the retardation of protein absorption since a positive effect is found only in easily digestible proteins.

The true digestibility of the protein was not found to be affected when the N-free diet was replaced by protein, fat, cellulose or lactose. These observations are of particular interest as several other workers have suggested the source of the N-free fraction to have a pronounced influence on TD values.

Processing of foods or feedstuffs by means of heat may affect protein quality – much depending on the method of processing. Boiling grain in a surplus of water does not seem to have any serious effect, although acid labile amino acids may be disturbed. Autoclaving protein together with reducing substances can very easily disturb protein quality as indicated by BV. Pelleting, however, seems to have a moderate negative effect.

With regard to the cystine/methionine ratio, the present investigations show that cystine can constitute at least 50% of the methionine + cystine requirement. Furthermore the specific requirement for cystine is very low.

Comparison of the results obtained with rats and pigs has shown a close agreement in the case of TD values. The BV data could only be compared for 6 diets, but nevertheless indicated that the two animal species ranked the different diets in the same order, although the data from pigs were significantly higher.

Further investigations are required in order to compare data obtained with rats and domestic animals (and human beings). However, recent work by *Eggum et al.* (1969) and *Madsen et al.* (1972) shows that similar, general conclusions can be obtained in experiments with rats, chicken and pigs respectively. Therefore it is felt that the results obtained in the present work with rats must give information of interest in the nutrition of domestic animals.

CHAPTER XVI

Dansk sammendrag

Kapitel I

Indledning

Hovedformålet med det foreliggende arbejde har været at indarbejde en forsøgsteknik for rotter, der kunne muliggøre en anvendelse af disse dyr ved belysningen af ernæringsfysiologiske spørgsmål vedrørende vore husdyr. Begrundelsen herfor er indlysende, idet omkostningerne ved at benytte rotter som forsøgsdyr er relativt små.

Det foreliggende arbejde omfatter i første række undersøgelser over proteinstoffernes kvalitet samt nogle faktorer, der kan påvirke proteinets udnyttelse. Som vurderingsgrundlag er benyttet *Mitchell's* metode (1924a), der angiver proteinstoffernes biologiske værdi. Denne metode har det fortrin fremfor mange andre metoder, at den inkluderer en række observationer, som samtidig gør det muligt at beregne proteinets sande fordøjelighed.

Proteinstofferne er i almindelighed ikke fuldstændig tilgængelige for organismen. Det samme er derfor tilfældet med aminosyrerne, da protein er opbygget af aminosyrer kædet sammen ved peptidbindinger. I det foreliggende arbejde er aminosyrernes tilgængelighed bestemt i en række fodermidler i forsøg med rotter og grise. Til dette formål er benyttet en metode udarbejdet af *Kuiken & Lyman* (1948) gående ud på at bestemme såvel de indtagne mængder aminosyrer som de mængder, der udskilles med gødningen. Da forsøgene er gennemført både med rotter og grise, er der foretaget en direkte sammenligning mellem forsøgsresultaterne fra de to dyrearter. Disse sammenligninger har bekræftet anvendeligheden af rotter som forsøgsdyr, hvorfor en række undersøgelser derefter blev gennemført med rotter alene. På grundlag af disse sammenligninger kan det således antages, at forsøgsresultater, der blev opnået ved forsøgene med rotter, også vil være af værdi, når det gælder ernæringen af vore husdyr.

Kapitel II

Den biologiske værdi som et mål for proteinkvaliteten

Den biologiske værdi af et proteinstof angiver den procentdel af det absorberede kvælstof, som udnyttes i organismen. Begrebet »biologisk værdi« som et mål for proteinets kvalitet blev først indført af *Thomas* (1909), men er senere blevet genoptaget af en række forskere, først og fremmest af *Mitchell*

(1924b).Metoden er baseret på kvælstofbalancer målt under klart definerede forsøgsbetingelser og omfatter foruden målinger af kvælstof i foder direkte målinger af kvælstofmængden i gødning og urin samt måling af stofskifte-kvælstof og endogent kvælstof. Stofskifte-N og endogent-N er i de foreliggende undersøgelser bestemt ved at give forsøgsdyrene et proteinstof, som udnyttes fuldstændigt (frysetørret, æterekstraheret ægprotein), d.v.s. hvis sande fordøjelighed og biologiske værdi er 100%. Stofskifte-N og endogent-N vil da være den mængde kvælstof, som findes i henholdsvis fæces og urin.

Mitchell's metode er blevet kritiseret på grundlag af, at aminosyrebehovet til vedligehold adskiller sig fra aminosyrebehovet til vækst. *Mitchell* (1959) var dog af den opfattelse, at aminosyrebehovet til vækst i det store og hele er det samme som til vedligehold. Forsøg tyder dog på, at lysinbehovet til vedligehold (*Mitchell & Beadles* 1950) er lavt i forhold til behovet til vækst. Methioninbehovet til vedligehold er derimod relativt højt (*Fisher et al.* 1960), og dette kan forklares ud fra, at methionin er den vigtigste metyldonator i det intermediære stofskifte. Til trods for disse ulemper ved *Thomas-Mitchell's* metode bliver resultater opnået ved denne metode i stor udstrækning benyttet som referenceværdier ved indarbejdelse af nye metoder (*Finlayson & Baumann* 1956, *Sheffner et al.* 1956, *Eggum* 1970d, *Rølle & Eggum* 1971).

Labile proteinreserver i organismen har hidtil været anset for at være af uvæsentlig betydning ved BV-målinger (*Holt et al.* 1962). Nyere undersøgelser (*Young* 1970) viser imidlertid, at treonin- og lysinkoncentrationer i muskelvævet er af en anselig størrelse – og således kan have en vis stødpudekapacitet. Dette forhold bør derfor tages i betragtning ved fastsættelse af forperiodens længde, idet BV kan blive målt for højt, hvor en af disse aminosyrer er den begrænsende.

Som en kontrol på kvælstofbalancemetoden har mange forskere foretaget en bestemmelse af forsøgsdyrenes kvælstofindhold efter aflivning, idet kvælstofindholdet skal modsvare det, der bestemmes ved *Mitchell's* metode. Mange finder gennemgående højere værdier for aflejret N ved *Mitchell's* metode sammenlignet med kropsanalysen (*Jakobsen et al.* 1960, *Henry* 1965, *Davidson & Williams* 1968, *Bønsdorff Petersen* 1969, *Just Nielsen* 1970), medens andre også finder overensstemmelser mellem metoderne (*Becker & Harnisch* 1958a, *Becker & Harnisch* 1958b, *Sanslone & Squibb* 1962, *Nehring et al.* 1964, *Buraczewska et al.* 1969).

Kapitel III

Forsøgsteknik for rotter

I dette kapitel er der foretaget en beskrivelse af balanceburene. Disse er oprindelig konstrueret af *Schiller* (1960) og *Horszczaruk & Bock* (1963). I hvert forsøg benyttes fem Wistar hanrotter, og forsøgstiden inddeles i en 4 dages

forperiode og en 5 dages balanceperiode. Hvert dyr får tildelt 150 mg N og 10 g tørstof daglig i såvel forperioden som balanceperioden.

Foderets kvælstofindhold indstilles ved hjælp af en N-fri blanding, der hovedsagelig består af kartoffelstivelse, der har været opvarmet til 120° C i autoklave.

Rotterne vejes ved forsøgets begyndelse og inddeles i hold à 5, således at holdgennemsnittene ikke afviger mere end ± 0,5 g. Rotternes vægt ved forsøgets begyndelse er ca. 75 g.

Gødning og urin opsamles i hele balanceperioden i Erlenmeyerkolber tilsat henholdsvis 100 og 50 ml 5% svovlsyre. I disse samleprøver bestemmes kvælstofindholdet ved Kjeldahlanalyse. Herved kan den totale mængde kvælstof udskilt i henholdsvis gødning og urin beregnes.

Stofskifte-N og endogent-N bliver som tidligere nævnt bestemt ved at give forsøgsdyrene et proteinstof, der udnyttes fuldstændigt. Til dette formål er benyttet et foder indeholdende 4% frysetørret, æterekstraheret ægprotein. Stofskife-N er proportionalt med den fortærede tørstofmængde og er bestemt til 2,04 mg N/g tørstof, medens endogent-N er målt til 15,2 mg pr. rotte/dag. Endogent-N er uafhængig af såvel fortæret fodermængde som dyrenes vækst i det variationsområde, hvori disse undersøgelser er udført. Aminosyresammensætningen i stofskifteproteinet er målt, og det fremgår af disse undersøgelser, at dette protein har et ret højt indhold af essentielle aminosyrer og følgelig må være af høj biologisk værdi.

Kapitel IV

Forsøgsteknik for grise

De benyttede stofskiftebure er de samme som tidligere beskrevet af *Ludvigsen & Thorbek* (1960). I hvert bur er der plads til fire pattegrise. Den anvendte forsøgsteknik er også meget nær den samme, som den der blev benyttet af de nævnte forfattere. I forsøgene er der hovedsagelig benyttet ornegrise af dansk landrace. Grisene blev fravænnet 10 dage gamle og blev først sat på forsøgsfoderet ved en alder af ca. 30 dage. Der indgik fire grise på hver forsøgsbehandling, og der blev foretaget målinger på hver gris i 3 balanceperioder i aldersintervallet 30–55 dage. Kvælstofindholdet i forsøgsfoderet blev indstillet ved hjælp af et N-frit foder, der hovedsagelig bestod af majsstivelse og rørsukker. Alle diæter blev ikke afprøvet ved samme kvælstofkoncentration, idet koncentrerede fodermidler blev givet afstemt med 3,0% N i tørstoffet, kornarterne afstemt med 1,5% og blandingerne afstemt med henholdsvis 2,0 og 3,8% N. Årsagen til disse varierende N-koncentrationer er af forsøgstek-

nisk art. Gødning og urin blev opsamlet 2 gange daglig og opbevaret for hele balanceperioden i samlebeholdere i kølerum ved 4°C. I disse samleprøver af henholdsvis gødning og urin blev der foretaget kvælstofanalyser.

Stofskifte-N og endogent-N blev hos grise bestemt på samme måde som hos rotter ved at fodre med 4% frysetørret, æterekstraheret ægprotein. Som ved forsøgene med rotter blev det fundet, at stofskifte-N er positivt korreleret med den fortærede mængde tørstof (r = 0,90). Det blev ligeledes fundet, at også det endogene N var positivt korreleret med tørstofoptagelsen (r = 0,94). Da den fortærede tørstofmængde er direkte korreleret med legemsvægten, kan den nævnte korrelation skyldes dette forhold. En korrelationsberegning mellem legemsvægt og endogent-N gav da også en tilsvarende korrelationskoefficient (r = 0,95). I forsøgene med grise er dog såvel stofskifte-N som endogent-N sat i relation til den fortærede mængde tørstof. Det blev fundet, at aminosyresammensætningen i stofskifteproteinet hos grise og rotter er meget nær ens.

Alderens indflydelse på proteinets udnyttelse blev undersøgt, og det fremgik heraf, at såvel kvælstoffets fordøjelighed som retention var uafhængig af dyrenes alder i intervallet 30–55 dage. Dette var tilfældet for både animalsk og vegetabilsk protein.

Kapitel V

Kort diskussion af metoder til beregning af aminosyrernes tilgængelighed

I litteraturen findes kun anført begrænset forsøgsmateriale, der tager sigte på at belyse de enkelte aminosyrers tilgængelighed. Dette skyldes antagelig, at en løsning af problemet er meget arbejdskrævende, samt at egnetheden af de benyttede metoder uden undtagelse er stærkt kritiseret. Der findes såvel kemiske som biologiske metoder. Ved så godt som alle kan man kun måle tilgængeligheden af en enkelt aminosyre ad gangen, hvorfor problemet nærmest synes uoverkommeligt. I nærværende undersøgelser til belysning af aminosyrernes tilgængelighed er benyttet metoden af *Kuiken & Lyman* (1948), hvor aminosyrernes tilgængelighed måles som proteinets sande fordøjelighed. Dette medfører således, at der må udføres aminosyreanalyser på foder og gødning samt på stofskifteproteinet. Metoden bliver dog ofte kritiseret på grund af den mikrobielle aktivitet i fordøjelseskanalen. Syntese eller destruktion af aminosyrer, der skyldes mikrofloraens aktivitet, vil resultere i henholdsvis lavere eller højere SF-værdier.

Kapitel VI

Mikrofloraens indflydelse på proteinets omsætning i fordøjelseskanalen

Som anført i kapitel V er metoden af *Kuiken & Lyman* (1948) kritiseret på grundlag af fordøjelseskanalens mikroflora. Der er ingen tvivl om, at mikrofloraen både deaminerer aminosyrer og syntetiserer bakterieprotein i fordøjelseskanalen. Spørgsmålet er kun, om denne aktivitet har nogen signifikant betydning på hele proteinomsætningen hos normale, sunde, enmavede dyr. Herom er meningerne meget delte. Forsøg med bakteriefri dyr sammenlignet med konventionelle kunne ikke give noget klart billede vedrørende mikrofloraens indflydelse på proteinets omsætning (*Nitsan* 1965, *Salter & Coates* 1971). Der hersker almindelig enighed om, at den mikrobielle aktivitet tiltager med dyrenes alder. Dette bekræftes dog ikke af *Giovanetti et al.* (1970), der ikke kunne måle forskelle mellem aminosyrernes sande tilgængelighed uanset om rotterne vejede 80 eller 300 g. Disse resultater er i overensstemmelse med undersøgelser af *Just Nielsen* (1968, 1971), der i forsøg med slagtesvin fandt, at aminosyrernes TF-værdi ikke blev påvirket af grisenes alder i vægtintervallet 20 til 90 kg levende vægt.

Forsøg med tilskud af antibiotika i foderet til reduktion af den mikrobielle aktivitet i fordøjelseskanalen (*Michel & Francois* 1956) viste, at aminosyrernes dekarboxylering blev reduceret forskelligt for de enkelte aminosyrer. Heraf måtte det forventes, at et antibiotikatilskud bevirkede, at aminosyrernes SF-værdi blev forskelligt påvirket, såfremt der eksisterer en udtalt effekt fra mikrofloraen. Forsøg med tilskud af såvel klortetracyklin som sulfatiazol til byg påvirkede ikke de enkelte aminosyrers tilgængelighed forskelligt, hvilket indicerer, at effekten af mikrofloraen på de enkelte aminosyrers tilgængelighed er minimal.

For at belyse om foderets N-fri fraktioner påvirker aminosyrernes tilgængelighed, blev aminosyrernes tilgængelighed i byg målt dels med bygprotein som eneste kvælstofkilde, dels i byg givet sammen med kasein i forsøg med rotter. Når byg anvendes som eneste forsøgsfoder, består foderets N-frie del hovedsagelig af stivelse fra byg, medens kasein + byg som forsøgsfoder bevirker, at der skal fortyndes med N-frit foder (kartoffelstivelse) for at opnå den ønskede N-koncentration i diæten. Aminosyrernes tilgængelighed i byg var dog ikke påvirket af denne ændring i foderets N-frie del.

Undersøgelser af *Erbersdobler* (1971) tyder på, at lagring af gødning kan medføre forandringer i gødningsproteinets sammensætning på grund af mikrofloraens aktivitet. Foreliggende undersøgelser viser imidlertid, at såvel rotte- som grisegødning kan opbevares i mindst 1 uge, uden at aminosyremønstret forandres. Der er endvidere anført analyser, der viser, at gødningsproteinets aminosyresammensætninger stærkt påvirket af foderproteinets sammensætning

til trods for sammenblanding af exogent protein med stofskifteprotein i gødningen. Aminosyrernes tilgængelighed målt i forskelligt varmebehandlet fiskemel viste, at samtlige aminosyrers tilgængelighed blev påvirket af behandlingen. Dette var også i overensstemmelse med et fald i SF af totalproteinet. Reduktionen i NPU med stigende varmebehandling af fiskemelet korresponderede med et tilsvarende fald i rotternes vægt ved forsøgets afslutning.

På grundlag af den refererede litteratur samt egne forsøg blev det fundet forsvarligt at fortsætte studiet af de enkelte aminosyrers tilgængelighed i fodermidlerne efter *Kuiken & Lyman's* (1948) metode.

Kapitel VII

De enkelte aminosyrers tilgængelighed samt proteinets kvalitet i femten fodermidler målt i forsøg med rotter og grise

For at opnå en større viden om de enkelte fodermidlers proteinkvalitet blev femten almindeligt anvendte fodermidler vurderet i forsøg med rotter og grise. Foruden aminosyreindholdet og aminosyrernes sande fordøjelighed blev proteinets sande fordøjelighed (SF), biologiske værdi (BV), nettoproteinudnyttelse (NPU) samt udnytteligt kvælstof (UN) målt. Der blev ialt undersøgt seks kornarter, seks koncentrerede fodermidler og tre foderblandinger.

Den sande fordøjelighed af total-N i byg, havre og rug ligger omkring 80, hvilket medfører, at omkring 20% af kvælstoffet passerer dyrenes fordøjelsessystem uden at absorberes. For milokorns vedkommende ligger fordøjeligheden lidt højere, idet henved 85% af kvælstoffet absorberes, og hos hvede og majs fordøjes endnu mere af kvælstoffet – ca. 90%. Kvælstoffet i kasein er fuldstændig tilgængeligt for organismen, medens fordøjeligheden af kvælstoffet i fiskemel ligger lidt lavere – omkring 93%. I kødbenmel absorberes ca. 87% af kvælstoffet, medens kvælstoffet i sojaskrå, jordnødskrå og solsikkeskrå fordøjes med ca. 90%. Kvælstoffets fordøjelighed i blandingerne var direkte afhængig af de enkelte proteinkomponenters fordøjelighed, således at man kan regne med fuldstændig additivitet, når det gælder proteinets fordøjelighed.

Fordøjeligheden af de enkelte aminosyrer er i de fleste tilfælde meget nær den samme som for total-N i de respektive fodermidler. Hos kornarterne fås dog generelt lavere SF-værdier for lysin, asparaginsyre, glycin og alanin sammenlignet med total-N, medens der blev fundet højere værdier for glutaminsyre, histidin og arginin. De lave værdier, der bestemmes for lysin, er særlig uheldige, da denne aminosyre er den begrænsende aminosyre i kornproteinet.

SF for de enkelte aminosyrer i de koncentrerede, proteinholdige fodermidler afviger gennemgående mindre fra SF for total-N end det er tilfældet med

kornproteinerne. I modsætning til de fundne resultater for kornprotein er der her ikke fundet lavere SF-værdier for lysinets vedkommende. I forsøgene med jordnødskrå og solsikkeskrå er der dog en tendens til, at lysinets fordøjelighed ligger lidt lavere.

Som allerede nævnt er lysin den begrænsende aminosyre i kornproteinet. Derfor kunne man forvente den højeste biologiske værdi i det kornprotein, som har det højeste lysinindhold. Dette er imidlertid ikke tilfældet, da havre har det højeste lysinindhold, medens rug med et lavere lysinindhold har den højeste biologiske værdi. Det synes uvilkårligt ulogisk, såfremt vi kun betragter den begrænsende aminosyre. Nyere undersøgelser af *Glem Hansen & Eggum* (1972a, 1972b) viser, at BV med stor sandsynlighed også påvirkes af de ikke essentielle aminosyrer samt af hele aminosyremønstret. Heraf fremgår, at BV ikke alene er afhængig af den begrænsende aminosyre.

Det fremgår af forsøgene med kornarterne, at BV er højere hos grise end hos rotter, og forskellen er større, jo lavere lysinindholdet er. Heraf kan man være fristet til at slutte, at rotter er mere lysinfølsomme end grise. Dette er dog ikke i overensstemmelse med de to dyrearters lysinbehov, idet *NRC* (1968) angiver, at grise har et større lysinbehov end rotter. Årsagen til forskellen ligger snarere i, at forperioden ved griseforsøgene har været for kort (3 døgn), således at den frie lysinreserve i blod og kropsvæv (*Young* 1970) kan have haft en vis supplerende virkning på det lysinfattige kornprotein i selve balanceperioden (4 døgn).

De fundne BV-værdier i forsøgene med koncentrerede, proteinholdige fodermidler til rotter svarer stort set til de værdier, der kunne forventes på grundlag af aminosyresammensætningen. En undtagelse er kasein, hvilket mere indgående er drøftet i kapitel XI. På grund af at der har været forskellige kvælstofkoncentrationer i foderet til henholdsvis rotter og grise, kan der ikke foretages en direkte sammenligning mellem BV-værdier for de to dyrearter.

Da NPU er en afledet størrelse, skal den ikke specielt kommenteres her. Det skal dog anføres, at NPU målt på rotter for byg, havre og rug er ca. 60, medens NPU i hvede og majs er ca. 50. Den laveste NPU-værdi er målt i milokorn, hvor der blev fundet en værdi på 44,3, d.v.s. at mere end halvdelen af kvælstoffet i milokorn udskilles gennem fæces og urin. De tilsvarende NPU-værdier målt på grise er alle højere, da BV er højere og SF stort set den samme for begge dyrearter. De højeste NPU-værdier blev fundet i kasein og fiskemel. NPU var her omkring 70. NPU-værdien for solsikkeskrå var 64,9, herpå fulgte sojaskrå og jordnødskrå med NPU-værdier på omkring 56, medens kødbenmel havde en NPU-værdi på kun 42,3. For blandingernes vedkommende lå værdierne højt, og her lå Rød laktal højest med en NPU-værdi på 70,7.

Det skal dog understreges, at proteiner med samme NPU-værdier meget sjældent vil have samme værdi i en blanding. Sojaskrå og jordnødskrå har

begge en NPU på 56, men det er velkendt, at protein fra sojaskrå, rigt på lysin og treonin, har en langt højere komplementerende effekt i en blanding end jordnødskrå, specielt når korn indgår i blandingerne.

UN er ligesom NPU en afledet faktor, og i beregningerne indgår der både en kvantitativ (N i % af tørstof) og en kvalitativ (NPU) faktor. I forsøgene med rotter blev der fundet en UN-værdi på ca. 1 for byg, havre, hvede og milokorn, medens den for rug og majs var henholdsvis 0,86 og 0,82. De lave værdier for rug og majs skyldes især det lave N-indhold. Kasein og fiskemel har begge meget høje UN-værdier (henholdsvis 10,92 og 9,04), d.v.s. at fiskemel indeholder mindst 9–10 gange mere udnytteligt kvælstof end kornarterne. Sojaskrå og jordnødskrå har næsten ens UN-værdier, henholdsvis 4,61 og 4,75, medens solsikkeskrå har en noget lavere værdi – 4,25 – og kødbenmel har en UN-værdi på kun 3,73.

For at sammenligne de resultater, der er fundet i forsøgene med henholdsvis rotter og grise, blev der udført regressionsberegninger på de enkelte kriterier fundet for de to dyrearter. Disse beregninger viste, at der er meget god overensstemmelse mellem SF-værdier fundet for henholdsvis rotter og grise med korrelationskoefficienter omkring 0,9. En t-test viste, at SF-værdierne bestemt på henholdsvis rotter og grise ikke er signifikant forskellige. Dette viser med andre ord, at resultater for SF af såvel protein som af de enkelte aminosyrer opnået i forsøg med rotter samtidig giver meget værdifulde oplysninger vedrørende grise.

For BV-værdiernes vedkommende er kun et ret lavt antal sammenlignelige mellem rotter og grise. Der blev beregnet en ret høj korrelationskoefficient (r = 0,86). Der var dog niveauforskelle med signifikant højere BV-værdier for grise.

Kapitel VIII

Indflydelsen af proteinkoncentrationen i foderet på proteinets udnyttelse

Der er almindelig enighed om, at proteinet udnyttes bedre ved et lavt proteinniveau i foderet end ved et højt. Dette er helt indlysende, hvis der er så meget protein i foderet, at det tjener som energikilde. *Miller & Payne* (1961) fandt således i forsøg med rotter, at der var en negativ korrelation mellem NPU og kalorier fra protein i foderet. I de herværende undersøgelser, hvor der er givet stigende mængder protein fra henholdsvis kasein og sojaskrå til rotter, blev der fundet en negativ korrelation (r = –0,99) mellem proteinindholdet og BV. TF derimod steg med stigende proteinindhold i foderet, medens SF var uafhængig af proteinniveauet. Heraf fremgår det, at BV og TF bør bestemmes ved samme proteinkoncentration i foderet, medens SF er uafhængig heraf.

Kapitel IX

Indflydelsen af foderets energiindhold på proteinets udnyttelse

Ved enhver proteinvurdering skal man sikre sig, at forsøgsdyrene tilføres tilstrækkelig energi fra den N-frie del af foderet. Herved sikres det, at proteinet ikke tjener som energikilde, men det kan i stedet udelukkende medgå til proteinsyntese. Der har imidlertid hersket nogen uenighed om, hvorvidt et ekstra højt energiindhold i foderet kunne have en proteinsparende effekt.

For at belyse dette spørgsmål er der ombyttet indtil 14% stivelse i foderet med fedt. Dette havde ingen indflydelse på hverken BV eller SF. Yderligere forsøg med protein af såvel høj som lav BV gav tilsvarende resultat – et højt energiindhold i foderet kunne ikke påvirke proteinets udnyttelse, uanset proteinets kvalitet.

Kapitel X

Indflydelsen af træstofindholdet i foderet på proteinets udnyttelse

Det er en almindelig opfattelse, at et stigende træstofindhold i foderet skulle have en negativ effekt på proteinets fordøjelighed. Den egentlige årsag til dette forhold formodes at være en stigende udskillelse af stofskifte-N med stigende træstofindhold i foderet (*Mangold & Behm* 1955, *Whiting & Bezeau* 1957, *Zorita & Schobinger* 1958).

Til belysning af træstoffets indflydelse på proteinets udnyttelse er der i forsøg med rotter givet stigende mængder cellulosepulver i foderet, indtil 30% af tørstoffet. Dette havde imidlertid ingen effekt på proteinets udnyttelse, idet såvel SF som BV forblev konstante uanset celluloseindholdet. Disse undersøgelser tyder ikke på, at stofskifte-N stiger med stigende træstofindhold i foderet. Derimod blev det fundet, at proteinet i fodermidler med et højt naturligt træstofindhold havde en lav fordøjelighed – men dette er ikke identisk med, at træstof som sådan har en negativ effekt på proteinets fordøjelighed.

Kapitel XI

Laktosens indflydelse på proteinets udnyttelse

En række undersøgelser tyder på, at laktose kan have en specifik virkning på proteinets udnyttelse (*Register & Petterson* 1958, *Maner et al.* 1962, *Buraczewski et al.* 1971). Den direkte årsag synes at ligge i, at laktosen kan påvirke fordøjelseskanalens pH (*Fischer & Sutton* 1949) og derved fordøjelseskanalens motorik. For at belyse spørgsmålene vedrørende laktosens eventuelle indfly-

delse på proteinets udnyttelse er der i forsøg med rotter givet tilskud af laktose til en række forskellige proteinkilder. Disse forsøg viste, at proteinets fordøjelighed var helt upåvirket af, om der var laktose i foderet. BV derimod var i enkelte tilfælde påvirket af laktosetilskuddet. Den positive virkning synes at gøre sig gældende, når der anvendes let fordøjelige proteinstoffer, idet BV i kasein, sojaskrå, fiskemel og æg øges signifikant på grund af laktosetilskuddet, medens de mere langsomt fordøjelige proteiner i byg og foderblandinger ikke påvirkes. Dette kunne tyde på, at absorptionen af proteinet kan foregå for hurtigt til, at der kan opnås maksimal udnyttelse. Dette er i overensstemmelse med undersøgelser udført af *Buraczewski et al.* (1971), der viser, at laktose hæmmer absorptionshastigheden.

Kapitel XII

Varmebehandlingens indflydelse på proteinets kvalitet

Det er velkendt, at proteinstofferne kan påvirkes af opvarmning, stærkt afhængig af under hvilke forhold varmebehandlingen foregår. De væsentligste faktorer, der påvirker resultatet, er temperaturniveauet og varmepåvirkningens varighed. Yderligere har produktets vandindhold samt tilstedeværelse af reducerende stoffer betydning. Varmepåvirkning af proteinstofferne kan være af vidt forskellig art (*Bender* 1970), men den mest kendte og vel også hyppigst forekommende er sikkert lysin-sukkerreaktionen (*Maillard* 1912). *Maillard*-reaktionen kan foregå selv ved ret lave temperaturer. Ifølge *Görnhardt* (1955) vil en stigning i temperaturen på 10°C bevirke, at lysin-sukker-reaktionens hastighed stiger til det firedobbelte. Heraf fremgår, at en eventuel fordelagtig effekt fra en varmebehandling hurtig kan resultere i en irreversibel skadepåvirkning. Ved stærk varmebehandling vil tilgængeligheden af adskillige – sandsynligvis af alle – aminosyrer sikkert blive påvirket (se kapitel V; *Ford* 1962, *Eggum* 1969b). Undersøgelser med byg (*Eggum et al.* 1969) viste, at tørring ved 100°C i 30–40 minutter bevirker et fald i lysinindholdet, SF og BV. Autoklavering af fiskemel medfører foruden et kraftigt fald i indholdet af lysin også et stærkt fald i indholdet af cystin, serin, treonin og asparaginsyre (*Mason & Weidner* 1964).

Som anført medfører tilstedeværelsen af reducerende stoffer, at faren for proteinforringelse øges under varmebehandlingen (*Carpenter & Ellinger* 1960, *Dammers* 1964, *Erbersdobler & Zucker* 1966, *Bujard et al.* 1967). Den frie ϵ-aminogruppe i lysin kan danne enzymresistente bindinger med de reducerende stoffer og derved gøre lysinet utilgængeligt for organismen. *Carpenter et al.* (1962) viste, at faldet i tilgængelig lysin er størst ved et vandindhold mellem 5 og 14%. Dette indicerer, at tørring ned til et vandindhold under

14% kun bør finde sted, når det er absolut nødvendigt. *Miller* (1956) fandt, at tørt materiale er ret varmestabilt, medens *Eggum* (1968a) fandt, at kogning i stort overskud af vand ikke øver nogen effekt på proteinkvaliteten.

Det korn, der benyttes til fodring af mink, skal helst underkastes en form for varmebehandling inden opfodringen (*Eggum & Jørgensen* 1971). Det blev derfor undersøgt, om kogning af kornet har nogen virkning på proteinkvaliteten. Korn og vand blev blandet i forholdet 1:3 og kogt i 30 minutter under konstant omrøring. De behandlede prøver blev sammenlignet med ubehandlede i forsøg med rotter. Aminosyreanalyserne viste, at visse aminosyrer, hovedsagelig de syrelabile som cystin, methionin og tryptofan, kan destrueres i et vist omfang under den anførte behandling. De biologiske kriterier påvirkes gennemgående kun lidt af varmebehandlingen. BV var dog signifikant lavere i den varmebehandlede byg sammenlignet med den ubehandlede, medens SF i havre steg signifikant, og det samme var tilfældet med BV for rugens vedkommende.

Autoklavering af fiskefars uden tilsætning havde ingen nævneværdig effekt på proteinkvaliteten, medens et tilskud af 5% kartoffelstivelse inden autoklaveringen bevirkede et fald i BV fra 85,7 til 71,3 – altså en stærk negativ effekt ved tilsætning af reducerende stoffer.

Pelleteringen af vore fodermidler foregår ved et lavt vandindhold samtidig med tilstedeværelsen af et stort overskud af reducerende stoffer (stivelse). Herved kunne der forventes en stærk negativ virkning på proteinkvaliteten, såfremt melet blev udsat for en betydelig varmebehandling under pelleteringen. Herværende undersøgelse viste, at lysinindholdet i svinefoderblandinger faldt 6–8% på grund af pelleteringen, medens de andre aminosyrer syntes upåvirkede. Dette fald i lysinindholdet medførte imidlertid ikke et signifikant fald i BV. NPU-værdierne var dog lavere i de pelleterede prøver end i melet, men kun i et tilfælde signifikant ($P < 0,05$).

Af denne diskussion fremgår det, at faren for proteinforringelse næsten altid er til stede ved behandling og lagring af vore fodermidler og fødevarer.

Kapitel XIII

Cystinets værdi som erstatning for methionin

Mitchell et al. (1968) fandt, at cystin kunne dække mindst 70% af det samlede behov for de svovlholdige aminosyrer til fravænnede grise. Dette er i god overensstemmelse med resultater af *Rao et al.* (1961), idet disse forskere ved forsøg med rotter fandt, at cystin kunne udgøre indtil 68% af methionin + cystin i foderet.

For at belyse spørgsmålet vedrørende erstatningsværdien af cystin for methionin blev der udført forsøg med rotter med tilsætning af begge disse aminosyrer

til fodermidlerne sojaskrå og kasein. Sojaskrå har et lavt indhold af både cystin og methionin, medens kasein kun har et lavt cystinindhold. Forsøgene med sojaskrå viste, at der blev opnået stor positiv effekt ved tilskud af såvel cystin som methionin, og at cystin kan udgøre mindst 50% af behovet for de svovlholdige aminosyrer. I forsøgene med kasein blev der opnået samme BV, uanset om der blev tilsat cystin eller methionin. Da indholdet af cystin i kasein er meget lavt (0,58 g/16 g N), indicerer dette, at behovet for cystin må være lavt, hvilket medfører, at foderblandinger med et lavt indhold af de svovlholdige aminosyrer kan beriges med methionin alene uanset det relative indhold af cystin.

Analytical methods

Nitrogen is determined in feed, faeces and urine by the method of *Weidner & Jakobsen* (1962) and the antitrypsin activity according to a modified method of *Frölich* (1953). The amino acids are determined as described by *Weidner & Eggum* (1966) and *Eggum* (1968c).

Statistical methods

The reference book used in present work is *Snedecor* (1956).

List of abbreviations

N = nitrogen
AAC = amino acid composition
AAA = amino acid availability
TD = true digestibility of the protein
BV = biological value of the protein
NPU = net protein utilization
UN = utilizable nitrogen
RC = retention coefficient of the nitrogen
AD = apparent digestibility of the nitrogen
PER = protein efficiency ratio
»BV« = biological value of the protein when measured at different protein levels
s = standard deviation (or deviation from regression)
s_b = deviation of the regression coefficient
r = correlation coefficient

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NB: Abbreviations of journal titles are according to »List of Serial Publications Regularly Scrutinised for Nutrition Abstracts and Reviews«, by the joint Library of the Bureau and the Rowett Research Institute in Aberdeen, Scotland.