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From feed nitrogen to milk protein

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Summary

A survey is given of the various aspects of the N metabolism of the lactating cow. Attention is paid to microbial protein synthesis in the forestomachs, to feed protein which passes on into the duodenum undegraded and to the absorption of amino acids from the intestines into the blood.

Three types of research, aiming at improvement of the utilization of feed N, are surveyed:

1. studies on rumen metabolism and amino acid absorption in which, among other methods, cows with re-entrant duodenal cannulae are used;
2. studies on losses of N in the intermediary metabolism due to the use of N for maintenance, due to energy shortage and due to a suboptimal pattern of the absorbed amino acids;
3. studies on a more precise information on N requirements of lactating cows fed various rations, including NPN, with long-term feeding trials and N-balance experiments.

Foreword

In 1953 the author, still a student, had the privilege to attend Prof. Dr. Mulder's lectures on the properties of milk proteins. Professor Mulder not only presented well-known facts on the subject but also introduced his students to the fascinating world of research in progress where facts still have to be disentangled from conflicting and unprecise data. It was one of the reasons why the author chose a research job which, although concerning the lactating cow's physiology rather than dairy chemistry, proved equally interesting. It was thought appropriate for this 'Mulder issue' of *Netherlands Milk and Dairy Journal* to survey recent progress of research on a subject related to milk protein: the nitrogen metabolism of the lactating cow.

1 Introduction

The high-yielding dairy cow has an extraordinary ability to increase its

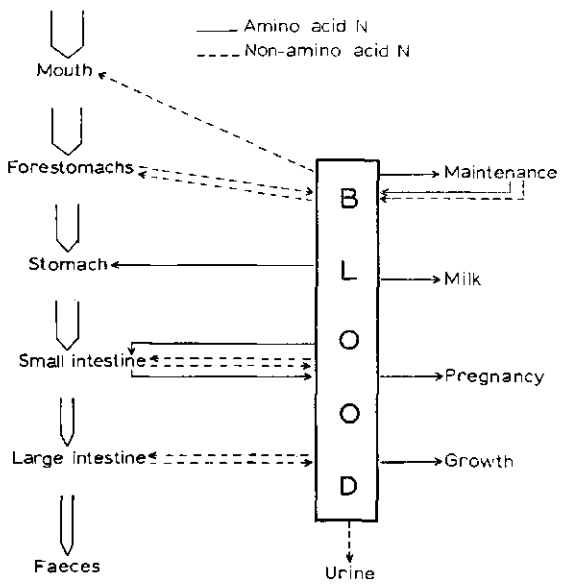

Fig. 1. Survey of N metabolism.

protein metabolism in the period around parturition. It uses daily about 0.3 kg absorbed protein to maintain itself when non-lactating and non-pregnant, and some cows produce as much as 1.5 kg milk protein daily a few months after parturition. The major part of the milk protein comes from the free amino acids of the blood, the remainder from blood peptides and proteins. As net synthesis of amino acids from non-amino acid compounds by the animal body itself is only possible to a limited extent, the required amino acids have to be supplied to the blood by absorption from the intestinal tract and, in cases of shortage, from the tissues after hydrolysis of tissue proteins. Inter-conversion of amino acids is impossible for the essential ones, but may occur for the others, in the udder as well as elsewhere (13).

It will be clear from this that the rate of absorption of amino acids from the intestinal tract is of the utmost importance for milk protein synthesis (Fig. 1). This is even more important because amino acids absorbed from the tract may also be used for other purposes. For instance, in case of energy shortage due to low feed intake, very low environmental temperatures or increased physical activity, amino acids may be used as an energy source. Secondly, in case of shortage of precursors for lactose synthesis and of reduced co-enzymes such as NADPH for fatty acid synthesis, glucogenic amino acids may be used. Finally, there is a continuous drain on the amino acids to maintain the tissues, for enzyme and hormone synthesis, tissue repair

and for the secretion of digestive enzymes into the digestive tract. Some of these amino acids return to the blood after their utilization, others are decomposed and lost.

In the ruminant most of the amino acids absorbed from the gastro-intestinal tract do not originate from the feed. Because of the activities of bacteria and protozoa in the forestomachs a considerable part of the feed proteins is hydrolysed and de-aminated and the resulting ammonia and non-protein nitrogen of the feed, if present, may be converted into microbial protein. Some bacteria may die and autolyse, others may serve as food for the protozoa. The degradation of feed protein is seldom complete. Some of the undegraded or partly degraded feed protein may reach the true stomach. Part of the ammonia of the degraded protein is often not converted into microbial protein. At higher levels some ammonia is probably absorbed through the rumen wall into the blood, rapidly converted into urea and excreted with the urine. Some ammonia passes on to the true stomach with the digesta. The digesta, furthermore, contain undegraded feed protein, feed NPN, bacterial and protozoal protein and some endogenous N (mucus, epithelial cells).

In the true stomach the hydrolysis of the proteins present is initiated and most of the protozoa decompose. In the small intestine the hydrolysis of the digesta is continued and (active) absorption of the amino acids takes place. Endogenous nitrogen, including the protein of the digestive enzymes, is secreted both into the true stomach and into the small intestine. The major part of the endogenous proteins is hydrolysed and most of the resulting amino acids are re-absorbed in the second part of the small intestine. The remainder of the digesta passes on into the large intestine where microbial activity again increases. Depending on the nutrients present, urea may enter the large intestine from the blood or ammonia may pass its wall into the blood. Absorption of amino acids, synthesized by the bacteria, into the blood very probably does not take place.

2 Some aspects of the efficient conversion of feed N into milk protein

As a considerable quantity of feed N has to be used for maintenance purposes, certainly at daily milk yields below 20 kg, the overall conversion of feed N into milk protein can be improved by high milk production levels and, furthermore, by avoiding unnecessary N losses in the faeces and urine. This is not only of advantage for the economy of the dairy farm but also for the world's supply of protein for human consumption. This kind of food protein supply is the more welcome because it is for a large part derived from plant material such as roughages which as such have hardly any value for human

nutrition. Much of the land used for growing these roughages, moreover, is not suited for crops which can be consumed by man. Moreover, especially in countries with a highly developed feed industry such as the Netherlands, even the concentrates used for dairy cows have not much value as human food, as they contain higher percentages of cellulose (6).

Obtaining high average milk production levels depends mainly on the genetic milk potential of the cow and on the supply of energy and N during the lactation, particularly in the first months (3). The highest efficiency of feed utilization will be obtained when the rations of the cows just supply the required energy and N, and not more, and when losses with excreta, heat included, are as low as possible. In this paper attention will be paid to the three main aspects of efficient utilization of feed N: (1) protein degradation and synthesis in the forestomachs and amino acid absorption from the small intestine; (2) losses of amino acids in the intermediary metabolism; (3) the N requirements of lactating cows.

2.1 Protein degradation and synthesis in the forestomachs and amino acid absorption from the small intestine

The bacteria and possibly also the protozoa of the forestomachs degrade most of the feed protein and feed NPN to ammonia, and may use this ammonia for synthesis of new microbial protein which can be degraded partly in turn. A net gain of protein occurs when more microbial protein is synthesized than feed and microbial protein is decomposed. Usually the benefit to the animal of this net gain is even greater because the amino acid pattern of the protein synthesized is closer to the pattern required by the cow for maintenance and milk and tissue synthesis (12, 8, 16, 17, 18).

The rate of growth of bacteria and protozoa depends mainly on free energy and N supply and on the time these microbes remain in the forestomachs. At 38 – 40 °C even microbes require a considerable amount of energy and N to maintain themselves. Additionally, energy is needed for growth, i.e. net protein, carbohydrate and fat synthesis. However, due to the anaerobic environment the supply of free energy, i.e. ATP, required for both purposes in the forestomachs is small. ATP is produced by conversion of feed carbohydrates and proteins into volatile fatty acids which yields many times less ATP than the complete oxydation of these nutrients. It will be clear that net bacterial and protozoal growth only occurs when more ATP can be produced than is needed for maintenance and sufficient building stones are provided for synthesis of protein, carbohydrate and fat. Stouthamer & Bettenhausen (15), working with anaerobic continuous cultures of bacteria, for this reason introduced the following model:

$$1/Y_{ATP} = 1/Y_{ATP}^{MAX} + m_e/\mu,$$

where:

Y_{ATP} = actual yield in g of dry organisms/mol ATP;
Y_{ATP}^{MAX} = Y_{ATP}, corrected for maintenance needs;
m_e = moles ATP needed hourly for maintenance per g dry organisms;
μ = specific growth rate of organisms.

Obviously high m_e and low μ values result in greater differences between maximal and actual yield of dry bacteria per mole ATP. The same applies for microbial protein production, since the major part of bacterial dry mass is protein. Therefore, much work is carried out with the aim of providing the bacteria and protozoa in the rumen with a sufficient and continuous supply of ATP.

A high rate of microbial activity in the forestomachs is only beneficial to the host animal if it increases the amount of energy (in chemical form) and amino acids absorbed into the blood. For this reason it should concern those feed components such as cellulose, which the animal itself cannot utilize because of the lack of appropriate digestive enzymes. Substances such as sugars, starch, fats and protein, which the host animal itself can digest in the small intestine, should preferably be left untouched. If they are nevertheless degraded, the resulting direct energy loss – with methane and fermentation heat – in this anaerobic environment averages only 10 – 20 % since the degradation is incomplete. The degradation products of these feed components as well as of feeds rich in cellulose are mainly volatile fatty acids which are readily absorbed into the blood and which are a good energy source for the animal.

Substances such as sugars, starch and soluble proteins are rapidly attacked by bacteria and degraded. This results in a rapid decrease of the pH of the rumen fluid because the acid production often exceeds the buffering capacity of the saliva which enters the forestomachs. At lower pH, especially below 5.5, microbial activity decreases. Thus less cellulose will be converted into volatile fatty acids when one or more severe falls in pH occur per day. As a consequence of such severe falls the energy supply to the host from cellulose is smaller. Thus, net microbial protein synthesis will be lower and passage of undegraded feed protein to the true stomach higher. The balance of these processes may be positive, zero or negative, depending on the circumstances. In the case of feed protein with an amino acid pattern which differs considerably from the pattern required for maintenance and milk protein, the decrease in microbial activity will be disadvantageous for the animal's amino acid supply.

At present, most research on rumen metabolism is concerned with finding simple means to direct microbial activity in the desired way: a high degradation of cellulose without too great breakdown of starch, sugars and proteins. A special case concerns the use of NPN instead of protein as part of the ration, in which case providing sufficient ATP sources for microbial growth is even more important. Of feed protein, a part usually reaches the duodenum undegraded and is a source of amino acids for the host. NPN can only become so after conversion into microbial protein.

Protecting some of the protein against microbial decomposition by coating with tannin or formaldehyde also helps the animal's amino acid supply but is expensive. Heat treatment of the feed has a similar effect. Both treatments, however, may be too severe, resulting in lower digestibility at the intestinal level. Feeding the concentrates more often per day, which reduces the incidence of falls in pH and thus enhances bacterial growth, is expensive but in highly automated farming systems might be possible.

Another method of changing the rumen microbial metabolism in such a way that it is of a greater benefit to the host animal is to alter the ratio of the end-products of fermentation. Czerkawski (4) used various methane inhibitors and succeeded in decreasing methane energy losses considerably. Nevertheless, microbial activity was scarcely depressed by the treatment since hydrogen atoms were diverted to microbial fat and protein, rather than to methane. Both from the point of view of energy and of amino acid supply this change is of advantage to the cow. Also addition of artificial saliva to the rumen (9) or its salts to the feed (19) seems to increase microbial growth. This is probably due to maintaining a higher pH as well as providing for a higher rate of flow of fluids with bacteria through the rumen. The better utilization of the metabolizable energy of rations containing ground roughages (10) may also be partly due to an increase in the production of bacterial protein, carbohydrate and fat. Salivation during and after the ingestion of such rations is low. The large surface area of the ground material suits rapid breakdown. Due to the lack of sufficient buffering capacity of the saliva, the pH soon becomes low. This decreases microbial activity but may at the same time divert it towards synthesis of propionate and bacterial protein, carbohydrate and fat, substances with a high nutritive value when present in the duodenum, rather than to CH_4, acetate and butyrate.

Cows equipped with re-entrant cannulae in the proximal duodenum are a great help for the study of the various conversions of the feed in the stomachs. Special analytical techniques which make use of particularities in chemical composition of bacterial and protozoal protein allow some subdivision of the N entering the duodenum with regard to origin. Unfortunately, these kinds

of investigations are very laborious.

The apparent absorption of amino acids from the small and large intestines into the blood averages 70 % of the amount entering the small intestine (see, e.g., 14, 17). This rather low value can be partly explained by the presence of D-amino acids in the cell walls of bacteria which are less easily absorbed than are L-amino acids. There are some indications that there is some preference with regard to the absorption of essential amino acids over non-essential ones (12, 18).

2.2 Losses of amino acids in the intermediary metabolism

Some of the amino acids of the blood are used *for maintenance purposes*, for example for enzyme and hormone production, and for tissue repair including protein turnover. It is difficult to measure the total quantity of amino acids required for these purposes but this is not a great disadvantage. Many of the amino acids of the proteins involved are not degraded any further but can be used again as such. Thus, it is only necessary to know the quantity of amino acids degraded during the process or lost with the faeces. Most of the nitrogen part of the amino acids degraded in the intermediary metabolism is converted into urea. The main path for the excretion of urea is by way of urine. Urinary nitrogen excretion is often used, especially in monogastrics, to estimate these losses. For this purpose either the animals are fed nitrogen-free rations, or extrapolations to zero nitrogen intake are made from urinary nitrogen losses determined at two or more levels of nitrogen intake. In the latter case the nitrogen intake should not be above the amount needed by the animal body. Any N consumed in excess of the needs will also be converted mainly into urea and excreted with the urine, so that the extrapolation does not give a reliable estimate of the net maintenance loss of nitrogen. Similar methods can be used for estimating the quantity of N voided with the faeces resulting from non-reabsorbed digestive enzymes, other secretations and sloughed-off epithelial cells. The quantity of this metabolic faecal N is related to the intake of dry matter. In fact, only the amount lost when a maintenance ration is consumed belongs to the maintenance metabolism.

In the ruminant, measuring maintenance N losses gives rise to even more problems. Our interest mainly goes to these maintenance losses during production, and it is not correct to assume that losses will be the same at high (up to 5 times maintenance, 40 kg milk per day) and zero milk production levels. This excluded all methods of estimation other than the extrapolation procedure. The lack of precise information on total nitrogen requirements also makes this procedure risky for estimating endogenous urinary N losses: more protein is easily supplied than is needed with the aim of avoiding de-

creases of milk yield. Moreover, in the ruminant some of the ammonia resulting from degradation of amino acids may leave the body with the faeces rather than with the urine or not at all. Part of the ammonia after conversion to urea returns to the forestomachs with saliva or via their walls and can be converted to microbial protein and absorbed as amino acids again. Urea may also pass through the walls of the intestines; part of it may be converted into microbial protein in the large intestine, but this time it will be lost with the faeces as very probably it will not be absorbed after hydrolysis to amino acids. Finally, the microbes of the end gut may decompose the N of enzymes, other secretions or epithelial cells to ammonia. Part of this ammonia may pass the gut wall to be excreted as urea with the urine instead of with the faeces. It will be clear that the interpretation of such extrapolation measurements in ruminants is very difficult.

Similar difficulties are met when the animal's *production metabolism* is studied. It depends on the pattern of the absorbed amino acids, their quantity and the requirement of the various amino acids for maintenance and milk production how much of these amino acids will be used. Unused amino acids will be degraded and most of the resulting N will be lost with the urine. Energy shortage will raise this N loss since amino acids are used as an energy rather than an N source in that case. Thus, sufficient energy should be supplied in experiments aimed at studying N requirements.

Amino acids may also be used to provide glucose for the synthesis of lactose and of the co-enzyme NADPH, needed during the synthesis of the higher fatty acids from acetic and butyric acid. Little glucose is absorbed from the gastro-intestinal tract into the blood of dairy cows. Most of the carbohydrates are converted into volatile fatty acids of which only propionic acid is a precursor for glucose. Glucose can also be used for synthesis of NADPH via the pentose-phosphate pathway. At milk yields above 20 kg the need for propionic acid for the synthesis of milk lactose and of NADPH needed for milk fat is often higher than the quantities of propionic acid absorbed. Thus, it was until recently assumed that glucogenic amino acids might be utilized as an additional supply. This again would increase the total requirements of absorbed amino acids of the lactating cow. Bauman et al. (1) recently demonstrated that in this animal species, which lacks other pathways of NADPH synthesis in addition to the pentose-phosphate route, NADPH can be produced in the cytosol by isocitrate dehydrogenase. Moreover, in the high-yielding cow, some glucose is absorbed from the gut (enclosed in bacteria or from feed due to rapid passage through the rumen), and some milk fat is made from the fat of the ration and microbes rather than from acetate. Together, it makes it rather unlikely that during protein shortage glucogenic

amino acids have to be used as a source of glucose and NADPH in the dairy cow.

It may be concluded that in the lactating cow it is very difficult to derive the precise amino acid requirements by accounting for all the separate parts of the N metabolism, i.e. by means of a factorial approach. Some attempts have been made to study this complicated part of animal metabolism with simulation techniques. Various rates of degradation of feed protein in the forestomachs, of synthesis of microbial protein and of absorption of amino acids from the small intestine were assumed to exist (11, 7). The effect of these assumptions of amino acid supply to the blood for different types of rations, also with NPN, fed at various levels was computed. It gave valuable information on the relative influence of the separate factors involved.

Some studies aimed at estimating the suitability of the amino acid pattern in the blood for synthesis of milk protein. Supposedly limiting amino acids were either infused in the blood or in the abomasum, or arteriovenous differences were measured over the udder. This work did not give much new information. This was partly due to technical difficulties, and partly to the fact that the pattern of the absorbed amino acids is usually not far from the desired pattern (13). Several analyses (8, 18) have shown that the amino acid pattern of protein of protozoa and, to a slightly less degree, of bacteria is close to the desired pattern for maintenance and milk production. Thus, only when much feed protein with a pattern different from that required passes on undegraded to the small intestine and is absorbed or when the bacterial protein has an exceptional amino acid pattern, is the pattern of the absorbed amino acids important. The first case may be found when much maize zein is fed which easily escapes microbial degradation, the second when NPN is the main N source in the feed and the levels of protein, minerals and vitamins are low.

2.3 N requirements of the lactating cow

In the preceding section we have seen that factorial methods to derive the N requirements of the lactating cow are still lacking in precision. For that reason, most requirement studies are done in a more direct way. Either long-term *feeding trials* or successive *N-balance trials* with lactating cows are used for the purpose.

In the first type of trial a few comparable groups of lactating cows are fed, for instance, 100 (control), 90 and 70 % of the amount of apparently digestible crude protein (DCP) assumed to be required for maintenance and production. The ration's content of DCP is usually computed from the composition of the ration or, occasionally, measured in a sheep digestibility trial

or with a few of the cows of the groups in a digestibility trial during part of the feeding trial. Milk yield and composition are the main criteria used to estimate DCP requirements. If yield and composition differ significantly from those of the control group, it is concluded that the lower DCP allowances are below the actual requirements.

Until a few years ago such trials were only performed with animals after the peak of the lactation's milk yield. A within-animal comparison in that case was partially made possible by enclosing the experimental period with low DCP by a fore- and after-period during which more DCP was fed. This made the interpretation of the results obtained less difficult. However, such a trial excludes the most critical period of lactation, the first eight weeks. Then feed intake, and thus N intake, especially in high yielding cows, is often not yet maximal. Moreover, to obtain a maximum milk yield during the whole lactation peak yield, the milk yield after 6 – 8 weeks, should be maximal (3). In view of this it is essential to include the first months of lactation in the trial, i.e. the experimental period of the trial should start around parturition. The absence of a fore-period with a sufficient N intake of the group with low N intake does not make the interpretation of such work easy. However, it approaches the situation at the dairy farm where for simplicity it is preferred to use one concentrate mixture with a constant N content throughout early and mid-lactation.

It will be clear that such studies should be repeated several times, with different types of rations and with animals having high and low milk potentials. They scarcely yield any information on the causes of the changes in milk yield found. Several such investigations have recently been started in the Netherlands.

The second type of study of N requirements consists of N-balance trials

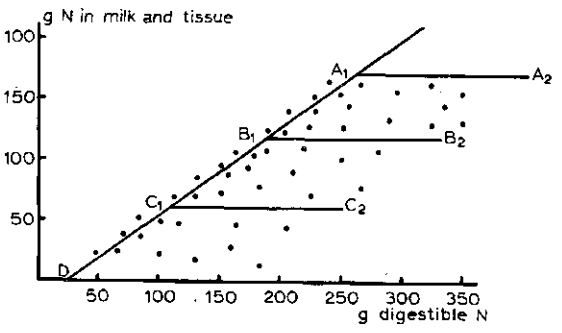

Fig. 2. Relationship between intake of digestible N and N deposition in milk and tissue. A_1-A_2, B_1-B_2 and C_1-C_2: N deposition at high, moderate and low milk yields. A_1-D: minimum requirement of digestible N for a given N deposition. Dots: results of N-balance trials with dairy cows in which just sufficient (dots near line A_1D) or excess digestible N (dots to the right of line A_1D) was fed.

with lactating cows. Several N balances have been measured as part of investigations on the energy metabolism of lactating cows (5). Also some N-balance measurements have been carried out to study the N requirements (2). N-balance studies last 3 to 4 weeks. All losses of ingested N with faeces and urine are measured quantitatively. Moreover, the N balance, i.e. feed N – faecal N – urinary N – milk N, gives an estimate of the amount of N deposited in or mobilized from the tissues of the animal. It should be mentioned, however, that all errors present in estimates of the separate elements feed N, faecal N, etc. accumulate in the estimate of this balance which therefore has not a high precision. The short period required for one trial allows the performance of many trials during the lactation of an animal, even in early lactation. Also, the effect of various N levels or of variation of the non-protein part of the ration can be studied within the same animals. N-balance trials, moreover, give a lot of information on several aspects of N metabolism such as the N distribution, on which a feeding trial does not provide many data.

So far the N-balance work (Fig. 2) has shown that the quantities of N required for maintenance and milk production are lower than was assumed so far. In addition, N stores in the tissues of the animal which could be mobilized during N shortage in early lactation were found to be small. However, these N-balance trials could not give, because of their short duration, any information on the long-term effects of low N supply on milk yield. It will be clear that both types of studies, balance and feeding trials, are needed for improving our knowledge of N requirements.

3 Final considerations

Many aspects of the N metabolism of the lactating cow are not yet clearly understood. Studies on rumen fermentation, investigations with cows with re-entrant duodenal cannulae, N-balance and energy-balance trials and long-term feeding trials are needed for a better understanding of the problem. The dairy cow is a fairly efficient convertor of feed which is not or only partly suited for human consumption into food which is highly suitable for that purpose. In view of this fact and the world's protein shortage it can be appreciated very much that several of the above-mentioned studies are in progress, also in the Netherlands, to improve the efficiency of the dairy cow's protein production even more.

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