STUDIES ON HOST ANIMAL PROTEIN AND ENERGY NUTRITION USING THE TECHNIQUE OF INTRAGASTRIC NUTRITION

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SUMMARY

Intragastric nutrition allows ruminant animals to be sustained without ingestion of food by infusion of volatile fatty acids (VFA) plus buffers into the rumen and of protein plus a mineral/vitamin mixture into the abomasum. The technique permits tightly controlled studies of many aspects of energy and protein nutrition.

The following topics are considered: utilisation and absorption of VFA mixtures; requirements for glucose and glucogenic nutrients in fasting and lactating animals; effect of clenbuterol on N balance; ability of undernourished animals to fuel lean growth from fat reserves; endogenous N loss, N secretion into the gut and purine metabolism; utilisation of microbial protein and the optimal composition of amino acid infusates for growth and lactation,

INTRODUCTION

Development of new techniques is often motivated by utter frustration and lack of progress using conventional techniques. In this respect the development of the technique of intragastric nutrition (IN) was no exception. The frustration occurred when it often became impossible to understand whether responses by the animal to nutritional manipulation was mediated through the rumen microflora or as a direct response by effect on the host animal. Changes in volatile fatty acid proportion and changes in the so-called metabolic faecal N serve as good examples of confusion of this kind as knowledge of these issues was required in order to make progress in new protein and new feed evaluation systems,

Attempts to sustain ruminants by IN have been made before but without sustained success. Our progress came with the ability to control rumen pH and the realization that VFA and buffer had to be added separately and that, due to VFA being absorbed mainly as the free acid, saliva was required only to neutralise less than 20% of the acids infused.

During the past decade a great deal of progress has been made in our understanding of host animal protein and energy nutrition through the application of our IN technique. Brief descriptions of the most important progress will be given below. Details of the technique itself are given by Ørskov et al. (1979) and MacLeod et al. (1984). Briefly, VFA and buffers are infused into the rumen and protein, trace minerals and vitamins into the abomasum. The animals normally adapt to this procedure during 2-3 weeks after which food is removed completely and the animals are sustained totally by IN.

ENERGY NUTRITION

Volatile fatty acid utilization

The ability to nourish ruminants totally by IN creates the possibility of varying the total energy input and the composition of that energy over a wide

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range. In the first study the acetic acid proportion was varied from 35 to 85 molar percentage with the proportion of propionic acid varying from 55 to 5 and the butyric acid held constant at 10 molar percentage. The results showed that heat production did not change over the range of VFA proportions used, thus demonstrating that the differences in utilization between roughages and concentrate could not be due to differences in the type of fermentation produced, Later work with cattle using an even greater range of VFA proportions has confirmed these observations. At an acetate proportion of 80% or more there is an increase in the blood β-hydroxybutyrate and *N* retention. At even higher proportions acetic acid is excreted in the urine (ørskov et al. 1979; KuVera et al. 1988; E.R. ørskov and N.A. MacLeod, unpublished). Determination of the energy cost of rumination, eating and other activities associated with roughage feeding can generally account for differences in utilization of roughages and concentrates.

Absorption of volatile fatty acids

The complete control of volatile fatty acid input also meant the controversy of whether molar proportions found in the rumen represented the VFA produced could be put to rest. MacLeod et al. (1984) showed very clearly that the relationships between the VFA proportions present in the rumen and those in the mixture infused could be markedly affected by rumen pH. Thus at a rumen pH of about 5.6 the molar proportions of acetic acid in rumen exceeded that of the infusate by more than 10 molar percent. At rumen pH of 7 or above the molar proportions in the rumen and in the infusate became similar. The results showed that at low rumen pH propionic and butyric acids were absorbed more rapidly than acetic acid and that the proportions found in the rumen do not necessarily reflect the proportions in which the volatile fatty acids are produced,

Fasting metabolism

While the achievement of a true fasting state in normally fed animals may take as long as 5 days due to the fermentation of feeds in the gut, a post absorptive state can be achieved in a few hours with IN. The most important observations made have been that the ruminant is clearly glucose-deficient during the state of fasting. This is indicated by an elevation of β-hydroxybutyrate in the blood and in an elevated excretion of N in the urine indicating use of tissue amino acids to generate glucose precursors (Asplund et al. 1985; KuVera et al. 1987). Infusion of small amounts of glucose (40g or equivalent of propionic acid in sheep and about 2-400 g/d in steers) is however sufficient to reduce β-hydroxybutyrate and urine N excretion to normal and basal levels, indicating that the requirement for glucose is very low. The dramatic effect on fasting metabolism of giving small amounts of glucose precursors also casts doubt on the use of fasting metabolism values in feed evaluation studies. The concept of differences in utilization of dietary energy below and above maintenance could be associated with abnormal metabolism during fasting. Infusion of glucose to otherwise fasted animals caused no increase in heat production and sometimes caused a decreased excretion of N in urine (ørskov, 1982).

Effect of type of nutrient on blood insulin and blood glucose

The ability completely to control the composition and level of nutrient infusion led to a study in which continuous or intermittent feeding has been simulated. About 20% of the total propionic acid used daily in the infusate

for dairy cattle was given as 3 h pulses during each 12 h to simulate the twice daily feeding of concentrate. This was compared with similar pulsed infusions of glucose and casein into the abomasum. While continuous infusions led to very stable plasma insulin levels over a wide range of inputs, pulsed infusions of propionic acid caused a rapid elevation of plasma insulin but no change in blood glucose. Glucose infusion elevated both insulin and blood glucose, while casein caused an elevation in blood insulin but a decrease in blood glucose (Istasse et al. 1987). Istasse et al. (1985) also demonstrated that dairy cows could be sustained by IN while yielding 20 or more kg of milk/day for a long period indicating that the nutrients infused contained all the vitamins, trace minerals etc. that the animal required.

Studies of growth control

We have also used sheep nourished by intragastric infusion to study the effects of adrenergic &agonists on nitrogen metabolism. The close control of nutrient supply possible, together with the fact that changes in nitrogen accretion are rapidly detectable by changes in the excretion of nitrogen in the urine, facilitate the use of within-animal comparisons of different treatments . The fact also that the excretion of nitrogen is virually all in urine enables measurements of protein turnover to be made relatively simply, since oxidation is represented by urinary excretion of nitrogen and therefore there is no need to use labelled carbon as the marker, This removes the problems of carbon recovery, and greatly simplifies the experimental approach,

We have found that the effect of clenbuterol on nitrogen retention is rapid; indeed, we can detect a fall in urinary nitrogen within 6 hours of starting an abomasal infusion of the drug (Herbert et al. 1985). The same work showed that the effects of the drug could be switched on and off. The drug increased N retention by 40%. There was some evidence of a slight carry over effect of the drug for about 1 day after it was withdrawn. Nitrogen gained was not lost when the drug was withdrawn. An interesting feature of these drugs is that their β-agonist effects on heart rate and plasma glucose are short term, lasting for less than 2 days, whereas that on nitrogen accretion persists throughout the treatment period.

These drugs also reduced basal nitrogen excretion (UN_E) of lambs on IN which received energy to maintenance (as VFA), but no protein. UN_E was reduced by about 20% on the second day of drug administration, but increased thereafter so that by the sixth day UN_E had returned to the level before treatment, Despite no detectable effect on nitrogen balance by day 6, there was a 12% increase in protein flux as measured by the irreversible loss rate of ieucine at this time, This contrasts with measurements which have been made on animals receiving an exogenous suppiy of amino acids in which most experiments have shown no change in protein synthesis, but a reduction in protein degradation,

Depletion/repletion studies

The IN technique is also well suited for measurement of depletion and repletion of both energy and nitrogen. Again, advantage is taken of the close and independent control which can be exerted over energy and protein supplies. In a series of experiments we have shown that with energy depletion (when protein was held constant, but was adequate) the animal could maintain itself in a positive nitrogen balance even when in negative energy balance (Hovell et al. 1983). These studies conformed well with the model of Black

and Griffiths (1975) which had not had wide recognition or application. These studies also showed that nitrogen retention was greater when the animal was on an ascending plane of energy supply than when on a descending plane of energy supply. When animals were depleted of protein and energy over a period of about one month, and then subsequently repleted, we found that with protein supplied greatly in excess of requirements, retention subsequent to depletion was much greater than before the depletion period (Hovell et al. 1987). This enhanced rate of accretion was maintained for a period of 3-4 weeks. The nitrogen lost during the period of depletion was replaced within a week, and therefore the enhanced accretion was true compensatory growth, and not simply repletion of protein lost, During the period of depletion, when protein was supplied at what had been calculated to be about 60% of maintenance, the nitrogen losses were substantially less than had been anticipated, If the efficiency of casein nitrogen utilization was assumed to remain constant at about 70%, then these results could be explained if it was assumed that the maintenance requirement for nitrogen had been reduced by about 30%. Such changes have parallels with differences in fasting energy metabolism in relation to previous nutrition as was demonstrated by Marston (1948). It is interesting that this adaptation in nitrogen metabolism appears to require an exogenous source of amino acids, for we have been unable to detect any adaptation in basal nitrogen loss with sheep on nitrogen-free diets. Clearly there is still a large area of study in protein/energy interactions in relation to previous nutritional history.

PROTEIN NUTRITION

Tissue maintenance requirement or endogenous N excretion

Determination of the maintenance requirement for protein has presented many difficulties in the past. Feeding of N free diets for any length of time was impossible since starvation of rumen microbes for N led to cessation of intake which invalidated the results, Extrapolation to zero of different levels of N intake gave similar erroneous results which were further complicated by erroneous concepts of the high metabolic faecal N in ruminants, The IN technique has made such measurements possible since the supply of protein can be stopped at will and the excretion of N then measured when no protein is given. The value adopted by ARC (Balch et al. 1984) of 350 mg $N/kgW^{0.75}$ was based on such measurements made in animals nourished by intragastric infusion (Hovell et al. 1983; Orskov et al. 1979; Storm et al. 1983a,b). The true metabolic faecal N was found to be about 35 mg/kg $W^{0.75}$ or about 10% of tissue maintenance requirements.

Using endogenous urinary N in the animal fed to maintain energy balance on the low-protein diet as the criterion for tissue N requirement, it has 'been generally assumed that the microbial protein produced would be far in excess of tissue requirement. However, use of the IN technique has shown this assumption to be erroneous. In fact, when animals are fed below energy maintenance there is a decline in protein status.

We have made a few measurements also of basal nitrogen loss during pregnancy in sheep. Pregnancy did not affect the total nitrogen loss, which was the same in pregnant and non-pregnant sheep and was the same also in pregnant sheep before and soon after parturition, Excretion of purine derivatives was, however, greater during pregnancy (M. Yule, X.B. Chen, F.D.DeB. Hovell and E.R. Orskov, unpublished).

Utilization of microbial protein

While it seems a paradox to use the IN technique to study rumen microbial protein utilization, this was the only way in which supply could be adequately controlled. Storm and Orskov (1983) managed to isolate about75 kg of dry rumen microbial matter from normal ruminants and this was used as the sole protein source for lambs on IN, thus enabling the efficiency of utilization of rumen microbial protein to be determined in ruminant animals for the first time. The content of amino acids in the microbial N was similar to that observed by previous authors. The average digestibility of amino acid N in the small intestine was 0.85 but there were differences in digestibility between the amino acids (Storm et al. 1983b) notably cystein which was considerably lower than the rest. The efficiency of utilization of microbial N was 0.53 in young growing lambs and 0.80 when it was expressed as efficiency of utilization of absorbed amino acid N. These values have been used in the protein scheme developed by ARC (Balch et al. 1984).

Optimum amino acid composition

Based on a new concept of determining limiting AA the IN technique is being successfully utilized to determine the optimum composition of supplement to microbial protein. The principle of this approach is first to determine the efficiency with which absorbed amino acids from the basal protein are utilized. As mentioned before the efficiency with which absorbed amino acid N from microbial protein is utilized was 0.80. Assuming that the efficiency with ideal protein could be 1.0 the full potential of utilization of microbial protein could theoretically be increased by $25\%, \frac{1.0-0.80}{0.80} \times 100$

This also implies that the same N balance could be achieved either by optimizing the AA composition or by adding 25% more of the basal protein. A mixture of amino acids identical in composition to the absorbed amino acids was added to supply an additional 25%, also by continuous infusion, The concept implies that with this supplement, the most limiting amino acid no longer limits the utilization of the basal mixture and that its removal from the supplement would result in N retention similar to the basal input alone. It also means that if each essential amino acid is removed in turn, any reduction in N balance will correspond exactly to the point at which that amino acid becomes limiting. This enables the optimum composition of the amino acid mixture to be calculated as well as the quantity required to supplement microbial protein for different types of livestock production. Such work has now been completed for growing lambs (Storm and Ørskov, 1984). and dairy cows (Fraser, 1988).

N utilization during undernutrition and consequences for body fat utilization

While the IN technique makes it possible to use N free infusion, it is of course also possible to supply protein alone, without supplementary energy. This was used to answer a question as to which protein given to fasted animals would be oxidized to supply ATP or utilized as a source of AA. Hovell et al. (1983) and Ørskov et al. (1983), showed clearly with calves; lambs, steers and dairy cows that protein was efficiently utilized and when protein was supplied above the tissue maintenance need the animals even attained positive protein balance. Thus body fat was used to fuel protein deposition and the oxidation of amino acids was minimised.. The practical implications of this phenomenon were demonstrated in a lamb fattening trial with comparative slaughter. Fattet et al. (1984) showed that lambs given straw diets supplying less than

their maintenance energy need could maintain or even gain in weight when supplemented with a source of undegradable protein. The lambs gained about5 kg in lean tissue weight at the same time as they lost 1 kg of fat during a 92 day feeding period. This has subsequently been confirmed in more practical type feeding trials with lambs and steers. Body fat can be used strategically to fuel protein deposition.

Urea metabolism and recycling

Another aspect of ruminant protein metabolism which has been investigated using the IN technique is that of urea recycling. Since rumen microbial activity is virtually eliminated under this system of feeding it was felt that this might allow us to identify those components of urea recycling which derive from the activity of rumen microorganisms and those which are specific attributes of host animal metabolism. Such experiments might also elucidate some of the control mechanism involved and indicate possible methods of manipulating urea recycling in the normally-fed animal. The remarkably stable 'steady-state' conditions which can be achieved in animals nourished by intragastric infusion also make this an ideal system for the application of kinetic techniques employing radioactive and stable isotopes. These methods, using mainly ^{14}C-urea have been used to measure urea synthesis and degradation in sheep nourished in this way and to examine the effect of changes in nutrient inputs and in sites of digestion on the transfer of urea from blood to gastrointestinal tract.

The initial experiments involved a comparison of sheep nourished by infusion or given dry feeds in the conventional manner (Whitelaw et al. 1988). This showed that there were no major differences in urea metabolism between the two methods of feeding. Surprisingly, the quantities of urea degraded were also similar under these two feeding regimes, indicating that an active rumen fermentation is not a prerequisite to the normal exchange of urea between blood and digestive tract.

The general picture to emerge from the various studies we have conducted is that the quantity of urea transferred to the GI tract in animals nourished by infusion is remarkably constant under a wide variety of dietary circumstances. For example, changes in total N input as casein ranging from 0.5 to 2.0 x the daily maintenance requirement of N had no significant effect on urea degradation. In other experiments, increases of 30% in total energy input, either as a standard VFA mixture or as butyric acid alone had no effect on the quantity of urea recycled to the gut (F.G. Whitelaw and J.S. Milne, unpublished). Animals nourished by infusion are known to retain a population of adherent ureolytic bacteria within the rumen epithelium (Cheng and Wallace, 1979; Dinsdale et al. 1980) and it seems likely that the urease produced by these organism is sufficient to maintain a normal rate of transfer of urea into the rumen even when an actively fermenting biomass is absent. Confirmation of this suggestion awaits the outcome of ^{15}N-urea infusion experiments but it seems likely also that NH_3 derived from thisureaissimply reabsorbed as such from the rumen without further metabolic transformation. Only when an active 'sink' of N-deficient microorganisms is present does the NH_3 become incorporated into larger molecules and so removed from the system; this can then result in urea being diverted from other sites of degradation and may also be reflected in a decrease in urea irreversible loss rate if adequate energy is available to allow utilisation of the additional microbial protein synthesized.

Purine excretion as a possible marker of production of microbial protein

The lack of progress in understanding how to manipulate rumen microbial fermentation as a source of protein for the host animal is at least in part due to difficulties of measuring microbial protein production. There are difficulties also in maintaining post-ruminally cannulated animals, in our ability to manipulate intake of such animals, and in separating microbial protein from other protein sources in abomasal and duodenal contents. The use of nucleic acids as a marker of microbial protein and in particular the use of the obligatory excretion of purine derivatives as allantoin has been suggested. However, the value of using urinary excretion was uncertain due to lack of information about the endogenous excretion of purine derivatives. The IN technique has now been applied to the determination of the endogenous excretion of purine derivatives (Fujihara et al. 1987b) and to the measurement of the relationship between infusion of microbial protein and excretion of purine derivatives. The method now shows great promise as an efficient means of determining microbial protein production in intact animals. It is particularly interesting that the endogenous excretion is about 3 times greater in the bovine than in the ovine (Fujihara et al. 1987a,b; Chen, 1989).

Minimal flow of endogenous N through the alimentary tract

While the endogenous N excretion into the gut is probably stimulated by foods passing through it was of interest to generate some minimal values. The mitotic index of rumen epithelial tisue was found to be similar in animals nourished by the IN method and normally fed animals. The fraction consisting mainly of sloughed epithelial cells from the respiratory tract, mouth, oesophagus and rumen wall is probably largely destroyed by rumen microbes under normal feeding conditions.

With N free infusions (Ørskov et al. 1986) the quantity of non ammonia N (NAN) leaving the rumen varied from 50 to 80 mg N/kg $W^{0.75}$ while that leaving the abomasum varied from 180 to 200 mg N/kg $W^{0.75}$. The amino acid composition of the NAN was similar to that of tissue protein except that it had a considerably higher cystine content probably reflecting keratinized tissue. It was of interest also that although no microbial fermentation occurred in the rumen the NH_3 concentrations in rumen fluid varied from 50 to 120 mg NH_3/l with N free infusion.

CONCLUSIONS

Although we have used the intragastric infusion technique to investigate a wide range of aspects of ruminant metabolism, we feel that we have hardly touched the potential the technique offers, Possible applications in research are limited only by our imagination, and our frustration now is not with the lack of a technique, but limited opportunities to apply it. With the technique we are now in the paradoxical situation for ruminant nutritionists of having better control over nutrient supply than do our colleagues working with monogastric animals. As with any tool, it has its limitations, but provided these are remembered, it offers the means of better understanding of many aspects of ruminant and mammalian metabolism.

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