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## LIVESTOCK IMPROVEMENT CORPORATION LECTURE

### Can protein utilisation from pasture be improved?

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#### ABSTRACT

Although temperate pasture plants usually contain sufficient protein to potentially support most types of animal production, this protein is utilised for ruminant production with relatively low efficiency because of the losses that occur via microbial deamination in the rumen. These processes are well understood, yet procedures that should theoretically improve protein capture, such as the addition of soluble carbohydrate or protected protein to the diet, are frequently not effective. The paper considers reasons for this situation. It also examines several additional reasons why plant protein utilisation may be impaired, such as: high rumen degradability, hepatic detoxification of absorbed ammonia and its associated costs in terms of amino acid catabolism, and the relationship between amino acid supply to the duodenum and tissue amino acid requirements.

The paper evaluates whether an enhanced supply of individual amino acids, or mixtures of amino acids, to the tissues might increase the production of meat, milk and wool. It also considers whether we have enough information to be able to assess the likelihood of using specific enhanced amino acid supply or novel combinations to manipulate product quality.

The efficacy of various mathematical models of protein digestion and metabolism are evaluated in relation to our current knowledge of the subject.

**Keywords:** pasture protein utilisation; production response; product quality.

#### INTRODUCTION

The dominant feature of New Zealand agriculture is that it is based on year round utilisation of fresh temperate pasture plants. Cost-effective management of this system provides the competitive advantage for our agricultural export industry. There are several limitations imposed by pasture feeding (Ulyatt and Waghorn, 1993); in particular protein, which is a major concern if value is to be added to ruminant products, is not utilised efficiently.

The protein in fresh temperate pasture has a number of special properties that determine its utilisation:

- It has one dominant protein, ribulose-1,5-bisphosphate carboxylase (Rubisco, Fraction 1), the enzyme responsible for carbon dioxide capture in plants.
- The amino acid composition of plant protein is very similar to that of the micro-organisms that digest it in the rumen (Ulyatt *et al.*, 1975), so the amino acid composition of duodenal digesta does not vary greatly in pasture fed ruminants.
- The protein in fresh pasture plants is 75-80% soluble (Mangan, 1982). Approximately 90% of it is degraded within one hour of entering the rumen (W.C. McNabb pers. comm).
- High protein degradability can lead to losses of up to 50% across the rumen at high pasture intakes (MacRae and Ulyatt, 1974; Thomson, 1982), which raises the possibility of insufficient protein reaching

the duodenum to meet the requirements of high producing animals.

- The protein and soluble carbohydrate contents of pasture vary throughout the year, so the diet is not always well balanced with respect to these nutrients.

#### PRODUCTION RESPONSES TO PROTEIN SUPPLEMENTATION

If, as suggested above, there is substantial loss of protein during rumen digestion and the animal receives insufficient or an unbalanced mixture of amino acids to meet production targets, we should expect a response to appropriate dietary supplementation with amino acids. A large literature exists on supplementation with proteins and/or amino acids that have been “protected” to some degree from rumen degradation (reviews: Merchen and Titgemeyer, 1992; DePeters and Cant, 1992). It would be fair to say that the results have been extremely variable: many positive responses and many cases of no response. Most of these experiments have been with dairy cows and few have used fresh temperate pasture as their base diet. The most consistent positive responses have been to casein supplementation. Of the individual amino acids used, by far the most emphasis has been on methionine and lysine. Generally, more consistent responses have been found to whole proteins or amino acid mixtures than to individual amino acids, suggesting that several amino acids are co-limiting (Metcalf *et al.*, 1996).

**TABLE 1:** Effect of protected protein supplementation on milk protein yield (g/d) in cows fed on pasture. (Control diet was pasture alone; UTP was unprotected casein. In the Carruthers and Penno 91995) experiment fish meal was fed at two levels; 150 g/d (UTP) and 300 g/d (TP)). Means within rows with different superscripts are significantly different (P<0.05) in those studies where statistical differences were expected.

Author	Dietary N (% DM)	Control	UTP	TP
Wilson (1970)	-	481 <sup>a</sup>	540 <sup>ab</sup>	554 <sup>b</sup>
		536	539	544
Rogers <i>et al.</i> (1980)	2.8	506 <sup>a</sup>	521 <sup>a</sup>	578 <sup>b</sup>
Minson (1981)	4.6	544	549	604
Brookes (1984)	3.6	-	810 <sup>a</sup>	820 <sup>a</sup>
Carruthers & Penno (1995)	-	760 <sup>a</sup>	780 <sup>a</sup>	790 <sup>a</sup>

**TABLE 2:** Production responses to protein in sheep fed pasture. (In all experiments the control diet was pasture. LWG is liveweight gain and Met is methionine).

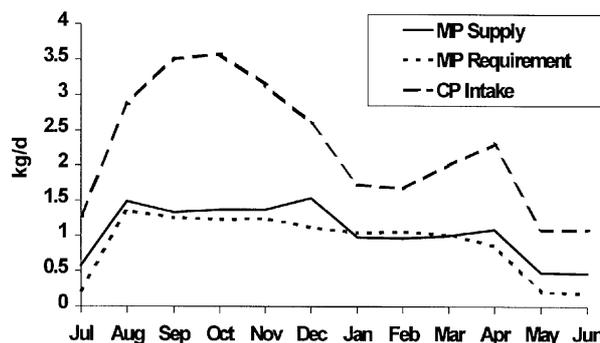
Author	Treatment	Animal	Production response
Barry (1980)	Abomasal casein + Met	Ewes	Reduced decline in lactation curve
Barry (1981)	Abomasal casein + Met	Lambs	25% > LWG, 69% > wool, 76% > carcass protein gain
Penning <i>et al.</i> (1988)	Fish meal	Ewes	24% > milk, 15% > lamb LWG, ewes lost less wt.

Results from supplementation experiments where fresh temperate pastures, similar to those used in New Zealand have been fed as the base diet, are presented in Tables 1 and 2. Supplementation of dairy cows with formaldehyde-treated casein gave a positive response in milk yield in two out of three cases and supplementation with fish meal gave no response in one experiment (Table 1).

Supplementation of sheep with various treatments (Table 2) had positive impacts on liveweight gain, carcass protein gain, wool growth and milk yield. Condensed tannin-containing legumes have been used frequently to protect protein from rumen degradation in pastoral systems. This subject has been reviewed extensively by Waghorn *et al.*, (1997). The most consistent positive responses have been obtained by feeding *Lotus corniculatus*, including increases in liveweight gain in heifers (Marten *et al.*, 1987) and sheep (Douglas *et al.*, 1995; Wang *et al.*, 1996b), increases in wool growth (Douglas *et al.*, 1995; Wang *et al.*, 1996b) and increases in ewe milk yield (Wang *et al.*, 1996a). Other condensed tannin-containing legumes have given very mixed results. It seems that responses to protein and/or amino supplementation in ruminants fed pasture-based diets have also been extremely variable.

The evidence suggests that a production response will be obtained only if the animal is truly limited in protein and/or amino acid supply, or it has surplus available energy at the tissue level. It is far from clear whether an animal fed a protein-adequate diet will respond to either heroic doses of additional protein or amino acids, or to biologically unusual mixtures of amino acids. This is

**FIGURE 1:** Simulation of the protein requirements of dairy cows grazing a Waikato pasture using the FeedTECH model (McCall and Ulyatt, unpublished). MP is metabolisable protein and CP is crude protein.



possibly a fruitful target for future research, although there are difficulties which will be discussed below.

Why have the production responses to protein and/or amino acids been so variable? Several reasons can be advanced:

- If a diet already provides the animal with its full amino acid requirement it is unlikely to respond to a supplement. A simulation of metabolisable protein supply and requirements for a dairy cow grazing typical Waikato pasture is shown in Fig. 1. In this case supply exceeded requirement for most of the year and the only time protein supplementation would be likely to produce a response was in January and February. Supplementation at other times of the year are likely to be unsuccessful. Fig. 1 also demonstrates a large difference between crude protein intake and metabolisable protein supply, which is a measure of the inefficiency of protein utilisation in cows fed fresh pasture.
- The base diet fed will have a large influence on any response to a supplement. This is nicely demonstrated in the data of Rogers *et al.*, (1979), where cows were fed either pasture silage or fresh pasture and were given an abomasal infusion of casein. Casein infusion greatly increased the yields of milk and protein, and milk protein % (P<0.01) in the cows fed silage, but only slightly increased milk yield and milk protein % (P<0.05) in the cows fed pasture. Many of the supplementation experiments reported in the literature will have failed because the base diet given would have supplied sufficient protein to satisfy requirements.
- The site of supplementation may have an effect on the result achieved. Infusion of supplement directly into peripheral blood is more likely to be effective than administration into the abomasum or the duodenum, and this in turn is likely to be more effective than administration via the feed or the rumen. Any supplement administered via the abomasum or duodenum is subject to modification during splanchnic metabolism, while any feed or rumen supplement must survive both rumen digestion and splanchnic metabolism.

- The experimenter needs to be sure that the method used to protect feed or rumen supplements is effective in delivering protein to the duodenum. For example, heat treatment is commonly used to protect protein concentrates such as fish or meat meals, yet commercial preparation of such meals frequently lacks quality control resulting in either undertreatment or overtreatment. Beever *et al.*, (1976) demonstrated that severity of heat treatment can have a profound effect on degree of protection. Formaldehyde treatment of protein sources is also commonly used to afford protection. Ashes *et al.*, (1984) showed that treatment with less than 500 mmol formaldehyde/kg protein provided incomplete protection in the rumen and that treatment with greater than 600 mmol formaldehyde/kg protein rendered a significant portion of the protein indigestible in the small intestine. Heat and chemical treatment are but two of many methods that have been used for increasing protein flow to the duodenum in ruminants: others include coating (usually with lipid), genetic modification of plant protein, stimulation of microbial protein synthesis (eg. addition of soluble carbohydrate) and reduction of microbial plant protein degradation. All need rigorous checking of their efficacy.

It is necessary to be extremely rigorous in interpreting the results from protein supplementation experiments. If a positive response was obtained to supplementation it is reasonable to conclude that the protection method was effective. However if there was no response it is essential to be sure that the protein source was truly protected and that the animal's nutritional environment was such that it could respond to a supplement, before the treatment can be dismissed as being ineffective. There are likely to be many experiments reported in the literature where the protein supplement was either underprotected and thus partially digested, or overprotected and thus not available to the animal. In either case the experimenter would not get a satisfactory response to protein supplementation.

### AMMONIA DETOXIFICATION

Fresh temperate pasture is frequently of high protein and energy digestibility (>80%), yet it rarely supports the level of production that its digestibility would suggest. For example, fresh grass is not utilised as efficiently as dried grass made from it (Raymond, 1964). This is partly due to the heat treatment of dried grass protecting dietary protein from rumen degradation, but there are also interactions in protein and energy digestion and metabolism that may cause inefficiencies. This can be seen in the results of Ekern *et al.* (1965) who measured energy metabolism in sheep fed the same grass either fresh or dried. The fresh grass had a higher metabolisable energy (MJ/kgDM), but drying reduced heat production to the extent that the dried grass had a higher energy retention and was used with greater efficiency for fattening than the fresh grass. There were also differences in the way protein was digested and metabolised: significantly less N was excreted in faeces and significantly more N was excreted in urine in sheep

fed the fresh grass. Greater urinary excretion of N was thus associated with higher heat production in sheep fed fresh grass. The extensive conversion of protein to ammonia that occurs in the rumen of animals fed fresh pasture is a major loss in its own right, yet there appear to be additional metabolic costs associated with the absorption of large amounts of ammonia. There is an energy cost of up to 4% of metabolisable energy intake associated with the synthesis of urea in the liver (Waghorn and Wolff, 1984), but a potentially greater cost in detoxifying excess ammonia. Lobley *et al.* (1996) have found, in studies using sheep fed lucerne pellets, that hepatic removal of ammonia appears to require the net utilisation of considerable amounts of absorbed protein. This second penalty would result in a lower proportion of absorbed amino acids being available for muscle and milk protein synthesis. Blood urea concentrations in ruminants fed highly digestible fresh herbage (Egan and Ulyatt, 1989; Waghorn *et al.*, 1994) are considerably higher than those produced by similar intakes of dried feed (Lobley *et al.*, 1996), which would suggest that the metabolic cost of detoxifying ammonia on fresh herbage could be very high (Greaney *et al.*, 1996). It might be possible to modify plant protein, through breeding or molecular genetics, to reduce deamination in the rumen and thus increase the efficiency of utilisation of pasture plant protein.

### CAN THE QUALITY OF RUMINANT PRODUCTS BE MANIPULATED VIA NUTRITION?

So far this paper has only considered the possibility of increasing animal production through more efficient utilisation of pasture plant protein. However the present marketing drive is towards adding value to ruminant products. Most high value products from ruminant animals are protein-based (eg., meat, milk, wool, skins and refined products such as enzymes, hormones and specific proteins [eg., casein, lactalbumin]), and it is reasonable to ask whether their protein composition can be manipulated to meet specific customer requirements. The traditional wisdom is that this cannot be achieved by nutritional means, yet there is a small amount of evidence to suggest that possibilities exist.

Donkin *et al.* (1989) treated dairy cows with a supplement of rumen protected methionine and lysine and significantly increased the casein content of milk ( $P < 0.01$ ) and changed the ratios of casein protein fractions ( $> \alpha$ -casein). Similar results have been obtained in New Zealand in mid lactation (D. Pacheco-Rios, pers. comm.). Several experiments (Table 3) have shown that feeding rumen protected methionine to sheep can change quality parameters in wool, such as fibre diameter, sulphur content and tensile strength. Similarly, Barry *et al.* (1981) showed that abomasal infusion to lambs of casein enriched with methionine significantly increased the protein to fat ratio in the carcass. Such results show that there is potential to manipulate animal product quality by nutritional means. However a lot more research is required to confirm these preliminary findings. We do not know at

**TABLE 3:** Wool quality responses to protected methionine feeding.

Author	Animal	Production Response
Floris <i>et al.</i> 1988	Ewes	Wool length > 7% Wool diameter > 15.5% Torsion resistance < 8.4%
Lee <i>et al.</i> 1992	Rams	Wool growth rate > 28% Wool sulphur > 10%
Staples <i>et al.</i> 1993	Mixed sheep	Wool growth rate > 6-27% Tensile strength > 7-10%
Mata <i>et al.</i> 1995	Wethers	Wool growth rate > 4.3-37.9% Wool diameter > 2.3-9.3% Wool sulphur > 0.2-12.8%

present whether it is possible to change product quality by supplementing with amino acids because the relevant research has not been done. We do not know whether supplementation with unusual combinations of amino acids will change product quality. One of the problems is that this type of research is expensive, especially if dairy cows are the target animals. There are eight amino acids regarded as being essential for mature ruminants. The number of experimental treatments required to test all possible combinations of them is 255 and superimposed on this is an infinite number of concentration possibilities. This is why past research has concentrated on picking likely winners such as methionine and lysine. This is a difficult problem, but scientific research is all about doing what is thought of as being impossible. Plant molecular biologists have developed the ability to insert foreign proteins or unusual amino acid sequences into pasture plants, so the delivery vehicle for getting unusual rumen protected proteins into grazing ruminants is available. The problem is that ruminant nutritionists do not know what combinations of amino acids to recommend because the underpinning nutritional research has not been done. This situation is a commentary on past decisions on research investment priorities. A number of good overseas research groups are starting to address this problem, however it is unlikely that they will base their work on pasture fed ruminants.

### MODELS OF PROTEIN UTILISATION

One possible cost-effective method for evaluating the likely effects of varying combinations of amino acids on, say, milk protein composition, would be by using mathematical models of protein digestion and metabolism.

Many empirical decision-support models are available for rationing livestock (eg., CamDairy, INRation, Spartan). They are useful tools for doing the job for which they were designed. They can be used in experimental design by predicting animal production responses to protein supplementation (eg., Fig.1), but they are not sophisticated enough to predict responses to complex mixtures of amino acids. The Cornell Net Carbohydrate and Protein System (CNCPS) contains an empirical sub-model that predicts the supply of essential amino acids to the duodenum, the amounts of these absorbed, compares them with

amino acid requirements calculated by a factorial method, and predicts whether any amino acids are limiting. CNCPS does not represent the complexities of tissue amino acid metabolism and cannot predict with any certainty likely responses to the infusion of complex mixtures of amino acids. Another approach has been to develop detailed mechanistic models of one amino acid, such as that of France *et al.* (1995) for leucine uptake by the bovine mammary gland. The authors claim that this model is applicable to other amino acids, but provide no evidence that it could evaluate the supplementation of complex mixtures of amino acids on detailed milk composition. Recently Baldwin *et al.* (1997) have upgraded their mechanistic model of a lactating dairy cow (Baldwin, 1995) to include the digestion and metabolism of four groupings of essential amino acids (Phe+Tyr; Cys+Met; Lys; and by difference, the remainder). This model is an advance because it incorporates a facility for evaluating the effects of amino acid availability on casein and  $\alpha$ -lactalbumin synthesis.

Many of these models can be useful in predicting animal production responses to protein, and in the case of the more mechanistic models, simple mixtures of amino acids. However, because we do not yet fully understand in sufficient detail the nature of the biochemical and physiological processes involved in the determination of product quality, it is questionable whether any of the existing models are at this stage sufficiently sophisticated to be able to simulate realistically the complex interactions involved.

### CONCLUSIONS

There is tremendous scope for improving the efficiency of utilisation of pasture plant protein, however:

- Rigorous experimentation is required to determine unequivocally whether supplementation with protein, or complex mixtures of amino acids, can enhance the production of animals fed pasture.
- We have an incomplete understanding of the biochemical and physiological mechanisms that determine the quality of protein-based ruminant products. A considerable effort is needed to improve this situation and to test whether supplementation with complex, perhaps biologically unconventional, mixtures of amino acids can alter product quality.
- New Zealand agriculture depends upon the year round use of temperate pasture plants. It is doubtful whether anyone else has the incentive to do the research necessary to improve the utilisation of protein from this pasture.

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