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THE EFFECT OF DIETARY PROTEIN ON DIGESTION AND FERMENTATION IN THE GASTROINTESTINAL TRACT OF SHEEP¹

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Résumé

INFLUENCE DES PROTÉINES ALIMENTAIRES SUR LA DIGESTION ET LA FERMENTATION DANS LE TRACTUS GASTRO-INTESTINAL DU MOUTON. — L'étude présentée veut déterminer la teneur en protéine de l'aliment capable de rendre optimale l'efficacité de la digestion chez de jeunes béliers recevant une ration d'entretien. Pour évaluer l'influence de ces protéines sur la digestion dans le rumen et le tractus gastro-intestinal, les béliers porteurs de canules duodénales ont reçu des régimes (ensilage de maïs, caséine, vitamines, sels minéraux) contenant respectivement 6,7, 10,2, 15,1 et 19,2 % de protéine brute. La ration comportant 6,7 % de protéine diminue l'utilisation de la matière organique. Lorsque la teneur en protéine de la ration augmente, on observe une augmentation de la digestibilité des fibres (ADF). La ration à 6,7 % de protéine n'assure pas une bonne croissance des bactéries dans le rumen. Mais lorsque la teneur en protéines passe de 10,7 à 19,2 %, il n'y a plus d'augmentation correspondante de la croissance bactérienne comme le montrent les mesures du flux d'azote non ammoniacal de l'estomac et de la synthèse d'azote d'origine bactérienne du rumen. Quand les protéines de la ration dépassent 11,1 % (l'ammoniac du rumen est supérieur à 3,65 mg N/100 ml), il y a un gaspillage d'azote dans le rumen mesuré par la relation entre l'azote ingéré et le flux d'azote non ammoniacal d'origine stomacale. Dans les conditions de l'expérience, la fermentation dans le rumen et l'efficacité de la digestion ne sont pas améliorées lorsqu'on augmente les protéines alimentaires au-delà de 11 % (et que l'ammoniac du rumen excède 3,65 mg N/100 ml).

Dietary protein plays two roles in the nutrition of the ruminant. Firstly, it supplies a source of ammonia, amino acids and peptides required for

microbial fermentation in the rumen and secondly, it provides amino acids for the host's metabolism either from microbial protein synthesized in the rumen or from dietary proteins that pass through the rumen undegraded. The first role of dietary protein is important; it is a variable that is easily altered in the diet and it affects feed utilization, performance and health of ruminants. There have been some attempts to define the dietary protein or rumen ammonia level required for optimum rumen fermentation (Hume *et al.*, 1970; Satter and Slyter, 1974; Allen and Miller, 1976; Slyter *et al.*, 1979; Pisulewski *et al.*, 1981) but the available data is conflicting. The study reported here was designed to define the level of protein required in the diet or concentration of its major end product in the rumen, i.e. ammonia, that would maximize digestive efficiency.

Materials and Methods

Four crossbred wethers, 38-50 kg liveweight, were each fed one of four diets containing 6.7 (A), 10.7 (B), 15.1 (C) or 19.2 (D) % crude protein (CP) during four 28-day experimental periods. Each sheep was fitted with a rumen cannula and three of the sheep also had a re-entrant cannula (Ivan and Johnston, 1981) in the proximal duodenum. All four sheep were used in the determination of apparent digestion in the whole gastrointestinal tract. The three sheep with re-entrant cannulas were used to determine rumen

metabolites and digestion within the stomach. The sheep were kept in metabolism cages, fed hourly from a belt-type automatic feeder and had continuous access to water and cobalvanized iodized salt blocks. The ingredients and composition of the diets fed are shown in table 1. The diets were mixed at the start of the experiment and stored frozen until required, and samples were taken during days 15-25 of each period. They were fed to supply approximately a maintenance level of energy intake for each sheep.

Total collection of faeces was carried out during days 15-19 of each period. Faeces were pooled daily for each sheep and stored at -20°C . At the end of each collection period, faeces were mixed and sampled. A 24 h total collection of digesta from the re-entrant cannula commenced on the 21st day of each period. The procedures for collection, sampling and storage of digesta have been described previously (Veira and Ivan, 1981). On the 25th day of each period samples of rumen contents were collected from each sheep once an hour for four consecutive hours. The samples were strained through cheesecloth and 5 ml of the strained fluid was added to 1 ml of a 25 % solution of metaphosphoric acid, shaken and stored at -20°C until required. A large sample was taken from each sheep at the last collection time and 200 ml of the strained rumen fluid was used to separate bacterial fractions by differential centrifugation and washing following the procedure of Hutton *et al.* (1971). The bacterial samples were freeze-dried.

Table 1. — Composition of experimental diets.

	Diet			
	A	B	C	D
<i>Ingredients (as is basis)</i>				
Corn silage	89.5	89.5	89.5	89.5
Corn starch	9.2	7.1	5.1	3.0
Casein	...	2.1	4.1	6.2
Vitamin-mineral mix	1.3	1.3	1.3	1.3
<i>Chemical analyses (DM basis), %</i>				
Dry matter	39.7	40.6	40.5	40.3
Crude protein (N \times 6.25)	6.7	10.7	15.1	19.2
Acid detergent fibre	20.3	18.8	19.4	20.5
Organic matter	95.1	95.1	94.9	94.8

For the estimation of rumen volume and dilution rate (Warner and Stacy, 1968), 100 ml of a 10% solution of polyethylene glycol 4000 (PEG) was injected into the rumen of each sheep on the 26th day of each period and samples of rumen fluid were collected at 0, 1, 2, 4, 6, 11, 24 and 26 h thereafter. The samples were analyzed for PEG according to the method of Malawer and Powell (1967).

Dry matter (DM) of duodenal digesta and diets was determined by freeze-drying while CP was determined as Kjeldahl N \times 6.25. Organic matter (OM) in diets, digesta and feces was calculated as the loss in weight on ashing at 600 °C and acid detergent fibre (ADF) was determined in diets, digesta and faeces by the method of Goering and Van Soest (1970). Ammonia in digesta and rumen fluid was determined by the Berthelot colour reaction (Novozamsky *et al.*, 1974) and diamino-pimelic acid (DAP) was measured in bacterial and digesta samples after hydrolysis with 6 N HCl using a Beckman 121M amino acid analyzer. The volatile fatty acids in rumen fluid were determined by gas chromatography as described by Erfle *et al.* (1979).

The flow of bacterial N from the stomach was calculated from the N:DAP ratio in rumen

bacteria and the DAP concentration in duodenal digesta (Hutton *et al.*, 1971). The OM fermented in the stomach was estimated by correcting the duodenal flow for microbial OM, assuming that OM represented 80% of microbial DM (Smith, 1975).

The data was examined by analysis of variance techniques. In scrutinizing the results two patterns were evident, either a linear trend with increasing dietary protein (e.g., apparent N digestibility) or the lowest protein diet appeared different to the other three diets which were all very similar (e.g., OM digestibility). In both instances these trends were tested by using single degree of freedom contrasts (Steel and Torrie, 1960).

Results

The apparent digestibility of OM in the stomach and in the entire gastrointestinal tract (GIT) was increased when dietary protein increased from 6.7% (Diet A) to 10.7% (Diet B) but remained unchanged at higher protein levels (Diets C and D) (table 2). Only the decrease in digestibility in

Table 2. — Apparent digestibilities in the stomach and in the total gastrointestinal tract (GIT) and amount of organic matter fermented in the stomach of sheep fed diets with different crude protein levels.

	Diets				SEM	Significance of treatment contrasts	
	A	B	C	D		Linear	A vs B, C, D
Dry matter intake (g/day)	761	722	753	757	55.8
Apparent digestibility (total GIT) (%)							
Organic matter	72.8	79.1	77.6	78.9	1.80	...	*
Nitrogen	41.0	64.7	75.1	82.3	2.18	**	...
Acid detergent fibre	47.1	54.9	53.5	62.7	2.78	*	...
Apparent digestion (in the stomach) (%)							
Organic matter	55.7	61.1	61.2	62.6	2.72	...	NS
Nitrogen	-35.1	-5.0	23.0	34.8	4.36	**	...
Acid detergent fibre	43.0	56.8	53.7	59.7	6.55		...
Organic matter fermented in the stomach ^a (%)	65.1	74.6	73.9	74.9	1.91	...	**

* $P < 0.05$, ** $P < 0.01$.

a: Estimated by correcting duodenal OM flow for bacterial OM.

the GIT (72.8 vs 79.1, 77.6 and 78.9 %) was statistically significant ($P < 0.05$). However, when OM digestion in the stomach was corrected for the flow of bacterial OM to derive the percentage of OM fermented, then the value of 65.1 % for OM fermentation on the 6.7 % protein diet (A) was significantly different ($P < 0.01$) from those (73.9 to 74.9 %) observed with the other three diets. The apparent digestibility of N increased linearly ($P < 0.01$) with increasing dietary CP in both the stomach and entire GIT. The apparent digestibility of ADF was variable but in the GIT there was a linear trend ($P < 0.05$) for increased ADF digestibility as protein level increased. There was a similar trend in the stomach but the linear response was not significant ($P > 0.05$).

Table 3 shows the effects of dietary protein on various measures of N metabolism. With increasing protein there was a linear increase ($P < 0.01$) in rumen ammonia (0.24 to 9.57 mg N/100 ml). Non-ammonia N (NAN) flow relative to N intake decreased in a linear manner ($P < 0.01$) from 1.32 g NAN/g N intake on the 6.7 % protein diet (A) to 0.56 g NAN/g N intake on the highest protein diet (D). When NAN flow was related to DM intake, the flow was lower ($P < 0.05$) on the low protein diet (A), compared to the other diets (B, C, D) (1.40 vs 1.70, 1.69 and 1.72 g NAN/100 g DM intake). Bacterial yield

tended to be lower on the 6.7 % protein diet (A), but values were variable with no significant differences ($P > 0.05$) among diets.

The relationship between duodenal flow (g/g N intake) and dietary protein concentration, and rumen ammonia N are shown in figures 1 and 2, respectively. In both cases the linear decrease in NAN flow in relation to the independent variable (CP, fig. 1; rumen ammonia N, fig. 2) is well illustrated.

Rumen volume and dilution rate averaged 2.91 litres and 12.6 % per hour, respectively, for all diets and were not affected ($P > 0.05$) by dietary protein levels. Similarly, volatile fatty acids were unaffected ($P > 0.05$) by dietary protein levels and averaged 71.7 μ M/ml with molar proportions of 59.7 % acetate, 24.5 % propionate, 1.4 % isobutyrate, 9.5 % butyrate, 1.8 % isovalerate, 1.8 % valerate and 1.3 % caproate.

Discussion

In devising diets for ruminants, protein is needed to allow optimum microbial growth by meeting the requirement of microbes for N as well as providing a source of amino acids for the

Table 3. — Rumen ammonia levels, duodenal non-ammonia N flow and bacterial N yield in sheep fed diets with different crude protein levels.

	Diets				SEM	Significance of treatment contrasts	
	A	B	C	D		Linear	A vs B, C, D
N intake, g/day	8.16	12.36	18.2	23.26			
Rumen ammonia, mg N/100 ml	0.24	3.44	7.05	9.57	0.763	**	...
Duodenal non-ammonia N flow							
g/g N intake	1.32	0.99	0.71	0.56	0.034	**	...
g/100 g DM intake	1.40	1.70	1.69	1.72	0.102	...	*
Rumen bacterial N yield							
g/100 g OM disappearing in the stomach	2.13	2.75	2.60	2.51	0.467	...	NS
g/100 g OM fermented in the stomach	1.81	2.20	2.15	2.04	0.453	...	NS

* $P < 0.05$, ** $P < 0.01$, NS Not significant.

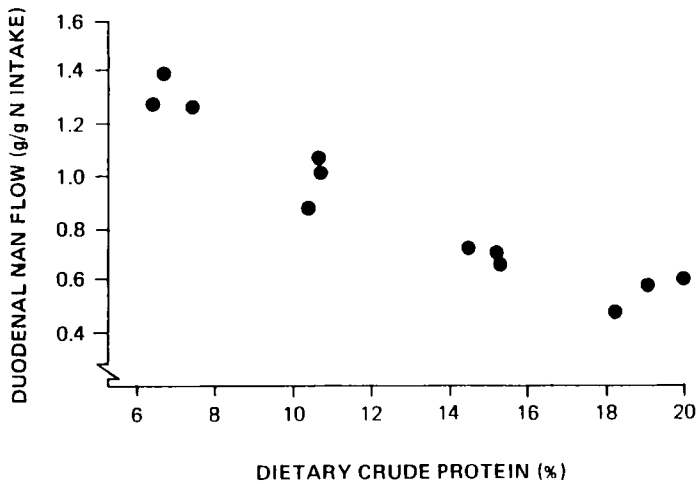


Fig. 1. — Relationship between duodenal flow of non-ammonia nitrogen (NAN) (g/g N intake) and level of dietary crude protein. Regression equation $y = 1.674 - 0.0606x$ ($r = 0.961$).

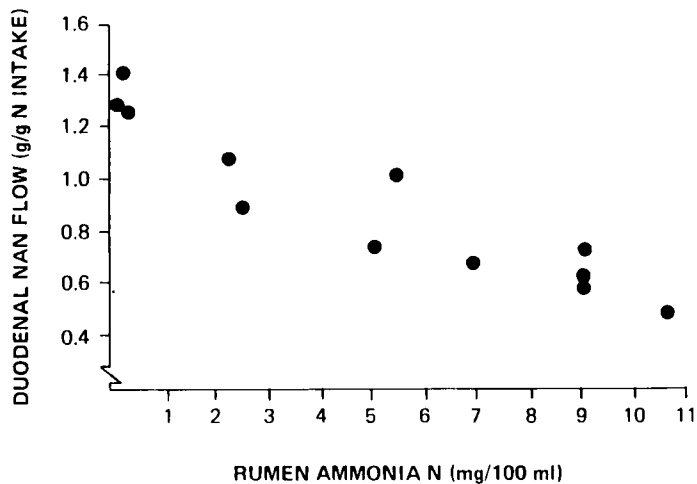


Fig. 2. — Relationship between duodenal flow of non-ammonia nitrogen (NAN) (g/g N intake) and concentration of ammonia N in rumen fluid. Regression equation $y = 1.27 - 0.0742x$ ($r = 0.934$).

host animal. In this paper the former aspect of protein nutrition in the ruminant has been studied. Other studies of this problem have used urea as a source of supplementary nitrogen (Hume *et al.*, 1970; Satter and Slyter, 1974; Allen and Miller, 1976; Pisulewski *et al.*, 1981). However, in this experiment casein was used to ensure that peptides, amino acids and ammonia would have become available to the rumen microbes during fermentation as these have been shown to be required sources of N (Pittman and Bryant, 1964; Maeng *et al.*, 1976). The similarity of duodenal flow of NAN (g/100 g DM intake) for the three diets in which casein was used at varying concentrations shows that the casein used in this trial was completely degraded in the rumen as has been observed in other experiments (McDonald and Hall, 1957; Hume, 1974). Thus any change in duodenal flow of NAN over that observed with 6.7 % CP diet can be attributed to a change in the microbial N flow rather than to changes in the flow of undegraded dietary protein.

In deciding what level of dietary protein is adequate for rumen fermentation several criteria could be applied. One criterion would be whether there was sufficient N for maximum microbial growth. From the flow of NAN (g/100 g DM intake) it was evident in this trial that 6.7 % CP diet was deficient and that levels greater than 10.7 % CP produced no increase. From the DAP-derived rate of bacterial synthesis there was a trend for the 6.7 % CP diet to result in a lower bacterial yield, though this was not significant. Since DAP does not measure the protozoa passing from the stomach, it is perhaps not surprising that the differences were not significant ($P > 0.05$). However, the fact that duodenal NAN flow (g/100 g DM intake) and bacterial N yield followed the same trend, provides evidence that for maximum microbial protein synthesis in the rumen, a dietary CP level between 6.7 and 10.7 % was required under the conditions of this trial.

The 6.7 and 10.7 % CP diets had corresponding rumen ammonia levels of 0.24 and 3.44 mg N/100 ml. *In vitro* estimates have indicated that rumen microbial needs are met when ammonia levels (mg N/100 ml) were 1.7 to 2.0 (Satter and Slyter, 1974) and 1.6 to 6.0 (Nikolic *et al.*, 1975). *In vivo* studies have reported optimum rates of microbial fermentation when ammonia levels (mg N/100 ml) were 8.8 to 13.3 (Hume *et al.*, 1970), 13.7 to 16.0 (Allen and Miller, 1976), 2.2 to 4.5 (Slyter *et al.*, 1979), ≤ 1.9 (Veira *et al.*, 1980) and 2.2 to 8.4 (Pisulewski *et al.*, 1981). The results

of this study are consistent with the above reports except those of Hume *et al.* (1970) and Allen and Miller (1976).

The variability between experiments in determining optimum dietary CP or rumen ammonia levels is probably due to differences in diet composition and level of feeding, the manner in which it is fed, the type of microbial population and the resulting pattern of rumen fermentation. Ishaque *et al.* (1971) and Pisulewski *et al.* (1981) have observed differences in rumen N metabolism when either an acetate or propionate type of fermentation developed. In a recent experiment (Veira and Ivan, unpublished) it was observed that the number of ciliate protozoa in the rumen had a significant impact on the efficiency of bacterial growth and so on N metabolism in the rumen.

A second criterion for deciding what level of dietary protein is optimum for rumen fermentation would be a measure of wastage of N in the rumen. The relationship between NAN flow and N intake (g NAN/g N intake) is a useful index of the efficiency with which dietary protein is used in the rumen. With the diets and level of feeding used in this experiment, there was wastage of dietary protein at levels greater than 11.1 % CP (fig. 1). The implication is that if more amino acids were required to meet the host animal's needs, then these would most efficiently be provided in a form that would not be degraded in the rumen but that would be digested in the small intestine. For rumen ammonia, the point at which NAN output from the stomach equalled N intake was 3.64 mg ammonia N/100 ml. The observations in this trial are similar to those reported by Veira *et al.* (1980) which were 12 % CP and 4.53 mg ammonia N/100 ml, but considerably less than the figure of 15-20 mg ammonia N/100 ml reported by Hogan and Weston (1970). This difference is possibly due to the nature of the diets used; Hogan and Weston (1970) were feeding grass and legume forages whereas in the present study and that of Veira *et al.* (1980) the diets contained considerable quantities of starch.

The final criterion for judging the adequacy of dietary protein is the level that maximizes digestion of the non-protein constituents. Judging by digestion of OM in the stomach and in the entire gut, the optimum level lies between 6.7 and 10.7 % CP, with 6.7 % being too low. In studies with corn silage supplemented with urea, Andrieu and Demarquilly (1974) observed that apparent OM digestion in the whole gut increased up to a dietary level of 8.5-9.5 % CP. When

urea was added to corn silage to increase the level of CP from 8.1 to 13.2 % Veira and Ivan (1981) observed no increase in the amount of OM fermented in the rumen of sheep. From the results of this trial and those reviewed, it would

seem that rumen digestion of organic matter is maximized when the level of dietary CP is between 8 and 10 %.

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Summary

Sheep fitted with duodenal re-entrant cannulas were fed diets containing 6.7, 10.7, 15.1 and 19.2 % crude protein (CP) to determine the effects of dietary protein in digestion in the stomach and in the entire gastrointestinal tract. There was a depression of OM digestion with the 6.7 % CP diet as compared to other diets. In contrast there was a trend for acid detergent fibre digestion to increase in a linear manner as dietary CP increased. From the flow of non-ammonia nitrogen (N) from the stomach and bacterial N synthesis in the rumen it was evident that the 6.7 % CP diet was too low for maximum microbial growth in the rumen, while there was no increase when protein level was increased from the 10.7 to 19.2 % level. From the relationship between non-ammonia N flow from the stomach and N intake or rumen ammonia it was observed that there was wastage of N in the rumen when dietary CP exceeded 11.1 % or when rumen ammonia was greater than 3.65 mg N/100 ml. It was concluded that with the type of diet and level of feeding used, rumen fermentation and digestive efficiency could not be improved by increasing dietary CP beyond 11 % or raising rumen ammonia beyond 3.65 mg N/100 ml.

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