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The Effect of Protein (as Fish Meal) on Rumen VFA Patterns of Molasses-fed Sheep

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RINGKASAN

Percubaan-percubaan in vivo *terhadap Kambing Merino yang diberi molasses telah dilakukan untuk melihat kesan meal ikan ke atas pengambilan molasses dan corak fermentasi asid lemak meruap (VFA) dalam rumen. Meal ikan diberi semasa rawatan yang berbalik. Perbezaan dalam pengambilan molasses disebabkan oleh protein dilihat dalam masa rawatan dan analisis regresi ke atas bahagian-bahagian VFA melawani molasses di masa rawatan menunjukkan bahawa tahap pengambilan adalah satu faktor pengatur dalam corak VFA yang terhasil. Propionat dan butirat didapati berkorelasi positif, manakala asetat berkorelasi negatif dengan pengambilan molasses. Kesan pengambilan dan nisbah tenaga/protein ke atas corak VFA juga dibincangkan.*

SUMMARY

Experiments in vivo *with molasses fed Merino sheep were carried out on a treatment reversal pattern examining the effects of fish meal (FM) supplementation on molasses intake and rumen volatile fatty acid (VFA) fermentation pattern. Differences in molasses intake due to protein supplementation were observed in the different periods. Regression analysis of VFA proportions against molasses intake during treatment periods indicated that the level of intake was a controlling factor in the patterns of VFA produced. Propionate and butyrate were found to be positively, while acetate was negatively correlated with the molasses intake. The effects of intake and energy/proterin ratio on VFA pattern are also discussed.*

INTRODUCTION

Rumen bacteria obtain their N for biosynthesis not only from NH_3 (Allison, 1969) but from amino acids and peptides (Pittmann and Bryant, 1964). With unconventional diets, such as those based on molasses (Preston *et al.*, 1967) or sugar cane (Leng and Preston, 1976) the protein content of the basic energy component is low and N is supplied as urea and supplementary protein. This supplementary protein may undergo digestion in the rumen and simply contribute to the NH_3 pool or it may be used directly by the micro-flora for growth or it may escape fermentation and undergo partial or total digestion in the abomasum and small intestine.

One of the common protein supplements which has been used with high sugar diets has been fish meal because of its low solubility and low rate of rumen degradation (Whitelaw and Preston, 1963). However the presence of some amino acids and peptides may affect microbial activity and can be detected in the patterns of VFA produced.

This paper describes the effects of addition or removal of fish meal on the daily ruminal fermentation pattern of molasses oaten chaff fed sheep.

MATERIALS AND METHODS

Three fistulated Merino wethers (about 30 to 40 kg) were kept in single pens with wooden slatted floors. They were adapted to either diet 1 (100 g oaten chaff fed once daily and molasses-urea fed *ad libitum)* or diet 2 (100 g oaten chaff with 60 g fish meal fed once daily and molasses-urea fed *ad libitum)* for about 10 weeks. The

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animals had free access to drinking water. Molasses contained 3% urea and 0.135% mineral mix.

During the period of study, the sheep were given the solid ration at 8 a.m. and about 20 mls or rumen liquor were withdrawn at 11 a.m. Each experiment lasted for about five weeks. The sheep were initially sampled for about a week on the adapted diet, then 60 g of fish meal was either given or withdrawn depending on the order of diet each one followed. Daily sampling was carried on for the next two weeks on this ration, after which the previous ration (with or without supplementation of fish meal) was started again and the sheep sampled for another two weeks.

Molasses intake was monitored by differences in the weight of containers. However, some losses from dribbling were unavoidable. The rumen samples were examined immediately for pH and then acidified. Volatile fatty acids were analysed by the method of Geissler *et al.*, (1976) and NH_3-N by steam distillation and titration.

RESULTS

pH

pH values of samples stayed within the range of pH 6.4 to 7.8.

Effect of Fish Metal on VFA Pattern and Nh_3–N

The result of VFA patterns for each sheep was considered separately as there were differences in the proportions of acetic (C_2), propionic (C_3) and butyric (C_4) acids. Table 1 shows the mean (± S.E.) molar proportions, of each held during the periods of with and without protein supplementation.

The results of Students's t-test applied to the differences in the means during the two treatment priods failed to show significant differences in fatty acid patterns attributable to the fish meal treatment.

Table 2 shows the mean (± S.E.) of NH_3-N concentrations (mg 100 ml^{-1}) of rumen samples. The mean values of NH_3-N of the three periods for each sheep were not significantly different.

Effect of Molasses Intake on VFA Pattern During Periods with and without Protein Supplementation.

Table 3 shows the mean (± S.E.) molar percentage values of C_2, C_3 and C_4 acids, and the mean (± S.E.) molasses intake for the three sheep respectively. When the molar % of each acid was regressed against molasses intake at different periods (+ fish meal and – fish meal), the majority of the relationships were not significant. However, there were indications of some significant correlations between the molar % of acetate, propionate and butyrate with molasses intake. Acetate was negatively correlated to molasses intake in one unsupplemented period (sheep 1) and during one supplemented period (sheep 2). Propionate was positively correlated to molasses intake in two supplemented periods (sheep 2 and 3) while butyrate showed a positive correlation with molasses intake in one supplemented period (sheep 1) and one unsupplemented period (sheep 3).

Effect of Fish Meal on Molasses Intake

Table 3 also shows the mean (± S.E.) of molasses intake for the different periods. Sheep 1 and 3 clearly showed differences in intake of molasses due to treatment. In the presence of fish meal, the amount of molasses consumed was significantly higher for sheep 1 ($p<0.001$) and sheep 3 ($p < 0.05$).

DISCUSSION

The pH values of rumen samples for these sheep are fairly high. Rumen pH of 6.4 has been observed in Holstein bulls fed high molasses by Reyes (1973).

The high rumen pH in molasses fed sheep is in contrast to the low pH observed when soluble carbohydrates are fermented (Eadie *et al.*, 1970). The high pH could be due to the way the animal consumed the molasses, that is, in small quantities throughout the day.

NH_3-H content of rumen liquor are slightly higher in the periods of fish meal supplementation. This could be partly due to urea as the levels of molasses-urea intake increased during this period.

For sheep 1 where the molasses intake was highest and consequently there was a low ratio of fish meal to molasses, the fermentation was predominantly high in butyrate in both the early periods but re-inclusion of fish meat led to a propionate rich pattern. At this period, molasses intake was significantly ($P < 0.001$) lower than the first period.

For sheep 2, with a much lower molasses intake and consequently a high protein molasses

TABLE 1

The mean (± S.E.) molar proportions of acetic, propionic and butyric acids during the periods of with and without protein supplementaion, and the result of Student's t-test applied to the differences in the means.

Acid	Sheep	Mean (± S.E.) molar % + fish meal	Mean (± S.E.) molar % − fish meal	t-test
Acetic	1	44.4 ± 3.5 (31)*	41.7 ± 2.9 (14)	
	2	54.3 ± 5.6 (14)	58.1 ± 3.5 (19)	NS
	3	57.7 ± 2.1 (20)	54.0 ± 1.9 (14)	
Propionic	1	24.0 ± 6.2 (31)	24.3 ± 3.4 (14)	
	2	31.1 ± 4.6 (14)	24.8 ± 4.1 (19)	NS
	3	33.4 ± 3.1 (20)	37.4 ± 2.5 (14)	
Butyric	1	29.3 ± 7.1 (31)	30.5 ± 5.6 (14)	
	2	12.8 ± 2.4 (14)	15.0 ± 2.9 (19)	NS
	3	6.7 ± 1.8 (20)	6.8 ± 1.2 (14)	

NS Not significant

* — Number of samples

TABLE 2

Means (± S.E.) of NH_3 –N concentrations (mg 100 ml^{-1}) for the three treatment periods of Sheep 1,2 and 3. The results of analysis of variance on the daily samples are shown.

Sheep	mg NH_3–N 100 ml^{-1} for treatment period															Analysis of variance
	+ fish meal				– fish meal				+ fish meal				– fish meal			
1	14.4	±	3.2	(8)*	13.9	±	2.8	(14)	14.0	±	1.6	(21)				NS between periods
2					9.8	±	3.5	(5)	16.3	±	1.2	(14)	12.2	±	1.3 (14)	NS between periods
3	18.8	±	4.5	(7)	15.2	±	2.0	(14)	18.1	±	1.7	(14)				NS between periods

NS – Not significant

* – Number of samples

TABLE 3
The mean (± S.E.) molar proportions of acetic (C_2), propionic (C_3) and butyric (C_4) acids of samples, the man (± S.E.) molasses intake and the relationship between molar percentages and the molasses intake for Sheep 1, 2 and 3.

Sheep	Period	No. of observations	Acid	Molar % (mean ± S.E.)	g Molasses intake day^{-1} (mean + S.E.)	Correlation between molar % and molasses intake
1.	+ fish meal	10	C_2	43.4 ± 1.3	1444 ± 137[a]	NS
		10	C_3	19.7 ± 0.7		Positive correlation ($r^2 = 0.47$, $P < 0.05$)
		10	C_4	34.6 ± 1.1		NS
	– fish meal	14	C_2	41.7 ± 0.8	1182 ± 52[b]	Negative correlation ($r^2 = 0.35$, $P < 0.05$)
		14	C_3	24.9 ± 1.1		NS
		14	C_4	30.8 ± 1.3		Positive correlation ($r^2 = 0.38$, $P < 0.05$)
	+ fish meal	21	C_2	45.0 ± 0.8	1105 ± 54[b]	NS
		21	C_3	25.8 ± 1.3		NS
		21	C_4	26.7 ± 1.5		NS
2.	– fish meal	5	C_2	59.9 ± 2.1	460 ± 31[c]	NS
		5	C_3	24.5 ± 1.3		NS
		5	C_4	14.3 ± 1.4		NS
	+ fish meal	14	C_2	54.0 ± 1.5	484 ± 34	Negative correlation ($r^2 = 0.41$, $P < 0.05$)
		14	C	31.2 ± 1.2		Positive correlation ($r^2 = 0.44$, $P < 0.05$)
		14	C	12.8 ± 0.6		NS
	– fish meal	14	C_2	57.9 ± 0.9	474 ± 28[c]	NS
		14	C_3	24.7 ± 1.3		NS
		14	C_4	15.3 ± 0.8		NS
3.	+ fish meal	6	C_2	57.8 ± 0.5	713 ± 62[d]	NS
		6	C_3	36.0 ± 1.2		NS
		6	C_4	4.8 ± 0.3		NS
	– fish meal	14	C_2	54.0 ± 0.5	696 ± 26[d]	NS
		14	C_3	37.4 ± 0.7		NS
		14	C_4	6.8 ± 0.3		NS
	+ fish meal	14	C_2	57.7 ± 0.7	811 ± 34[e]	NS
		14	C_3	32.2 ± 0.7		NS
		14	C_4	7.7 ± 0.4		Positive correlation ($r^2 = 0.34$, $P < 0.05$)

ab means without letter in common differ at $P < 0.001$

a b c d e means without letter in common differ at $P < 0.05$

NS – Not significant

ratio, the fermentations were proprionate rich compared to butyrate (with a higher percentage in the supplemented period).

For sheep 3, where molasses intake was about half way between the intake of sheep 1 and 2, the fermentation pattern was propionate predominant over butyrate.

It is interesting to note that the two periods in which propionate proportion rose significantly with molasses intake are both in protein supplemented periods. Sutherland (1977) suggested that the appropriate conditions for propionate being the preferred hydrogen acceptor would be under good conditions of microbial nutrition.

The two factors of molasses intake and protein to molasses ratio obviously do not cover all the variation in the VFA patterns observed. Animal factors are important. Sheep 1 and 3, although consuming a high amount of molasses and following similar feeding conditions, differed markedly in the predominant VFA patterns and obviously a great deal remains to be discovered on the factors controlling such patterns.

There are many reports in literature where addition of protein has led to increasing voluntary intake, particularly in the case of low quality roughage (Weston, 1967). The same applies with molasses where daily molasses intake increased with increased proportions of dietary nitrogen (as fish meal) in fattening bulls (Preston, 1972). The greater part of fish meal could be expected to escape rumen degradation due to its relative insolubility, which implies that the amount of amino acids entering the sites of metabolism play a determinant role in intake control.

CONCLUSION

Molasses intake and protein energy substrate ratios seem to be important controlling factors for the pattern of volatile fatty acids produced in sheep fed molasses urea.

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