

# The influence of dietary protein and fibre on bile-pancreatic secretion in sheep

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## ABSTRACT

Two experiments, each on 6 sheep of about 40 kg liveweight, fitted with a catheter in the common bile duct and a T-piece cannula in the duodenum, were conducted to assess the influence of fibre (Experiment 1) and protein (Experiment 2) in the diet on exocrine pancreatic and bile secretion. Three isonitrogenous diets made of meadow hay and concentrate containing 134 (LF), 200 (MF) or 313 (HF) g/kg crude fibre (Experiment 1) and three diets containing similar levels of fibre and 130 (LP), 170 (MP) and 200 (HP) g/kg crude protein (Experiment 2) were fed. Mean volumes of bile-pancreatic juice and total nitrogen content were not affected by the level of fibre and amounted to 1294 ml and 3.11 g/24 h, respectively. Mean volumes of secretions were higher ( $P<0.01$ ) on the HP than on the LP and MP diets. Total nitrogen was higher ( $P<0.05$ ) on HP (3.35 g/24 h) than on LP and MP (2.66 g/24 h). Total activity (U/24 h) of trypsin and chymotrypsin were not affected by the fibre level in the diet. Total activity of trypsin on the LP, MP, and HP diets was similar but chymotrypsin activity was greater ( $P<0.01$ ) on the HP than on the LP and MP diets. The amino acid composition of bile-pancreatic secretions was not affected by the diet composition.

**KEY WORDS:** sheep, diet, protein, fibre, bile-pancreatic secretion

## INTRODUCTION

Numerous studies have been conducted in different species to elucidate the physiological characteristics of exogenous pancreatic secretion. Activities of digestive enzymes produced by the pancreas are influenced by diet composition and are adapted to obtain effective digestive processes (Harada and Takeuchi, 1999).

In some non-ruminants high protein diets significantly increase the activity of pancreatic chymotrypsin and trypsin (Brannon, 1990) while in pigs, a high protein diet increases secretion of chymotrypsin, but trypsin secretion remains relatively constant (Corring, 1977).

Adaptation of pancreatic protein and proteolytic enzyme secretion to dietary composition in ruminants has been investigated less than in other species. Experiments similar to studies on monogastrics showing increased proteolytic enzyme secretion with increased protein intake are lacking for ruminants (Croom et al., 1992; Harmon, 1993). In ruminants with fully developed forestomachs, the composition of digesta entering the duodenum is less influenced by diet composition than in monogastrics. Ruminal fermentation is why microbial protein is the predominant source of protein entering the duodenum under most feeding conditions and its proportion in total protein of the digesta does not change very much. On the other hand, adaptation of pancreatic proteases responds only to the amount and composition of intestinal digesta protein (Brannon, 1990).

It was shown by Harada and Takeuchi (1999) that short chain fatty acids, which are the major end products of microbial fermentation of carbohydrates in the rumen, stimulate pancreatic enzyme secretion. This may suggest that different proportions of fibre or starch in the diet could result in a different output of enzymes in pancreatic juice.

The aim of the present study was to examine the influence of the level of fibre and poorly degradable zein protein in the diet on the volume of pancreatic and bile secretion, and the content of nitrogen on trypsin and chymotrypsin activity.

## MATERIAL AND METHODS

Two experiments, each on 6 Polish Merino male sheep of about 40 kg live-weight, were conducted in a 2 x 2 Latin square design. The animals were fitted with a silastic catheter into the common bile duct and a cannula into the duodenum distal to the papillae. In Experiment 1 the animals were fed isonitrogenous diets with low (LF), medium (MF) or high (HF) fibre contents (Table 1), and in Experiment 2 the sheep were given diets with low (LP), medium (MP) or high (HP) protein contents and a similar proportion of fibre (Table 2). Diets containing contrasted levels of fibre were prepared by including different amounts of meadow hay, and those with contrasting levels of protein were made by including different amounts of zein. The animals received 1 kg of feed per day in four equal portions given every 6 h (Experiment 1) and two equal portions given every 12 h (Experiment 2), with free access to water.

After 12 days of adaptation to the tested diets the bile-pancreatic juice was collected continuously for 48 h into bottles kept on ice. The weight of the juice

was measured every hour and 5% samples were taken and pooled for 6 h in Experiment 1 and for 12 h in Experiment 2. The remaining secretion was returned to the duodenum by means of a peristaltic pump at a rate similar to the outflow. Samples taken for analysis were stored at -70°C until analysis.

TABLE 1

Composition of the diets, g/kg

	Diets		
		<i>Experiment 1</i>	
	LF <sup>1</sup>	MF <sup>2</sup>	HF <sup>3</sup>
Meadow hay	400	700	950
Ground barley	495	200	-
Soyabean meal	105	100	50
Crude protein, g/kg	151	158	160
Crude fibre, g/kg	134	200	315
		<i>Experiment 2</i>	
	LP <sup>4</sup>	MP <sup>5</sup>	HP <sup>6</sup>
Meadow hay	600	600	600
Ground barley	400	400	400
Zein	-	40	90
Crude protein, g/kg	130	170	200
Crude fibre, g/kg	205	197	188

<sup>1</sup> - low fibre level; <sup>2</sup> - medium fibre level; <sup>3</sup> - high fibre level

<sup>4</sup> - low protein level; <sup>5</sup> - medium protein level; <sup>6</sup> - high protein level

TABLE 2

Mean volume of bile-pancreatic juice and amount of total nitrogen secreted during 24 h

Diets	Bile-pancreatic juice ml	Total nitrogen g	Total nitrogen % of intake
		<i>Experiment 1</i>	
LF <sup>1</sup>	1337	3.27	13.6
MF <sup>2</sup>	1324	3.09	12.9
HF <sup>3</sup>	1222	2.97	12.4
		<i>Experiment 2</i>	
LP <sup>4</sup>	1160 <sup>B</sup>	2.65 <sup>a</sup>	14.5
MP <sup>5</sup>	1292 <sup>AB</sup>	2.68 <sup>a</sup>	11.5
HP <sup>6</sup>	1398 <sup>A</sup>	3.35 <sup>b</sup>	11.3

<sup>AB</sup> P<0.01; <sup>ab</sup> P<0.05

<sup>1,2,3,4,5,6</sup> - as in Table 1

Total nitrogen and crude fibre in feeds and total nitrogen in bile-pancreatic juice were analysed using standard methods (AOAC, 1990). Amino acid analysis of juice was performed with a Beckman 6300 High Pressure Amino Acid Analyzer using modified procedures according to Buraczewska and Buraczewski (1981). Trypsin and chymotrypsin activities were estimated according to the method described by Hummell (1959).

The results were subjected to one-way analysis of variance using Statgraphics Plus ver. 7.0 (1993).

## RESULTS

In Experiment 1 the volumes of bile-pancreatic juice were similar during four consecutive 6 h periods and amounted to 331, 334 and 303 ml for diets LF, MF and HF, respectively. The mean bile-pancreatic juice output in 24 h did not differ significantly among diets LF, MF and HF (Experiment 1) but was significantly higher ( $P < 0.01$ ) after feeding the MP and HP diets than LP (Experiment 2; Table 2).

The mean content of total nitrogen was not significantly affected by the level of fibre and amounted to 3.11 g N/24 h (Experiment 1), but in Experiment 2 the total nitrogen content was significantly greater ( $P < 0.05$ ) with diet HP (3.35 g/24 h) than with diets LP and MP (2.65 and 2.68 g/24 h) (Table 2).

The total activity (U/24 h) of trypsin and chymotrypsin in bile-pancreatic juice was not affected by the level of fibre in the diet. The total activity of trypsin on diets LP, MP and HP was similar but chymotrypsin activity was greater ( $P < 0.01$ ) on diet HP than on LP and MP (Table 3).

The amino acid composition of bile-pancreatic juice, expressed in g/16 g N, was similar after feeding the animals all of the experimental diets (Table 4).

TABLE 3  
Total enzymatic activity (U) of trypsin and chymotrypsin in the bile-pancreatic juice secreted in 24 h

Diets	Trypsin	Chymotrypsin
	<i>Experiment 1</i>	
LF <sup>1</sup>	66 221	37 804
MF <sup>2</sup>	59 388	37 482
HF <sup>3</sup>	69 204	39 750
	<i>Experiment 2</i>	
LP <sup>4</sup>	52 011	29 082 <sup>Aa</sup>
MP <sup>5</sup>	58 548	34 357 <sup>Abc</sup>
HP <sup>6</sup>	56 138	39 937 <sup>Bb</sup>

<sup>1,2,3,4,5,6</sup> - as in Table 1

TABLE 4

Mean amino acid content in bile-pancreatic juice secreted in 24 h, g/16 g N

Amino acid	Experiment 1	Experiment 2
<i>Essential amino acids</i>		
Threonine	4.8	4.9
Valine	5.8	6.1
Isoleucine	4.0	4.0
Leucine	6.3	6.4
Tyrosine	4.7	4.8
Phenylalanine	3.1	3.2
Histidine	1.8	1.8
Lysine	4.6	4.8
Methionine	0.8	0.9
<i>Non-essential amino acid</i>		
Asparagine	9.5	9.7
Serine	6.1	6.7
Glutamine	7.8	8.3
Proline	3.4	3.5
Glycine	9.7	7.7
Alanine	4.4	4.5
Arginine	3.1	3.3
Cysteine	3.3	3.2
	<i>Total amino acid</i>	
	83.2	83.8

DISCUSSION

Pancreatic and bile secretion in ruminants may differ from that of monogastrics because of ruminal fermentation of fibre-rich diets, absorption of the end products and continuous postruminal flow of digesta. The digestive condition of ruminants differs considerably therefore from the posprandial, postabsorptive conditions of monogastrics. Taylor (1962) and Pierzynowski (1986) reported that pancreatic juice secretion in sheep fed standard diets is continuous with no or small changes after feeding, however, in cattle kept without feed for 48 h the volume of juice declined to about 50% (Pierzynowski, 1989).

According to Harada and Kato (1983), volatile fatty acids (VFA), which in ruminants are the main end product of carbohydrate microbial fermentation in the rumen, are potential stimulators of pancreatic exocrine secretion. They showed that butyrate, propionate and acetate given intravenously stimulated pancreatic juice flow, protein and amylase secretion under anaesthesia conditions.

In our study we did not find any effect of the diets containing different proportions of meadow hay and barley grain and therefore different amounts of fibre and starch on bile-pancreatic juice output, total nitrogen content and total trypsin and chymotrypsin activity. It is possible that under such dietary conditions, microbial fermentation, the rate of absorption and the proportion between particular VFAs absorbed did not differ enough to affect pancreatic secretion. In the presented experiment pancreatic juice and bile were collected together and the greater volume of bile than of pancreatic juice could mask small changes in pancreatic juice volume. On the other hand, similar values of trypsin and chymotrypsin activities for all three experimental diets suggest that under our experimental conditions differences in fibre content in the diets had no or only a small effect on secretion of pancreatic proteases.

Increasing the protein content in the diet from 130 to 200 g/kg by addition of zein significantly enhanced the amount of bile-pancreatic juice and total nitrogen secretions during 24 h, while increasing the protein content to 170 g/kg of the diet had no effect. Trypsin activity did not change with increasing protein content but chymotrypsin activity was significantly higher when protein was supplemented than on the control diet. Studies on pigs (Corring, 1977) and rats (Brannon, 1990) showed that pancreatic secretions and trypsin and chymotrypsin activities increased as the protein content in the diets rose, and chymotrypsin showed a more sensitive response to changes in protein consumption.

Nitrogen compounds entering the digestive tract with bile-pancreatic juice may play a significant role in the total nitrogen recycling system in sheep, which supports the results reported by Krawielitzki et al. (1999) who, using the <sup>15</sup>N dilution method in sheep, estimated that more than 9 g N/d of endogenous nitrogen was secreted into the stomachs and proximal duodenum, including pancreatic juice and bile. In the present study the amount of total N secreted in pancreatic juice and bile daily ranged from 2.65 to 3.35 g, which accounted for about 28% of that secreted into that part of the digestive tract, and from 11.3 to 13.6% of daily nitrogen intake.

## CONCLUSIONS

The fibre content in the diet for sheep has no influence on bile-pancreatic juice secretion or its amino acid profile, but a higher undegraded protein level in the diet increases nitrogen secretion and chymotrypsin activity in sheep.

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## STRESZCZENIE

**Wpływ poziomu białka i włókna w diecie na sekrecję żółciowo-trzustkową u owiec**

Przeprowadzono dwa doświadczenia, każde na 6 trykach o masie ciała około 40 kg z kateterami umieszczonymi w przewodzie wspólnym żółciowo-trzustkowym i prostą kaniulą w dwunastnicy. Zwierzęta żywiono w układzie kwadratu łacińskiego: w doświadczeniu I – dietami składającymi się z siana łąkowego i mieszanki treściwej, zawierającymi 134 (LF), 200 (MF) i 313 (HF) g/kg włókna surowego, a w doświadczeniu 2 – dietami zawierającymi 130 (LP), 170 (MP) i 200 (HP) g/kg białka ogólnego przy zbliżonej zawartości włókna. Objętość soku trzustkowego z żółcią i zawartość w nim azotu ogólnego nie zależała od poziomu włókna w dawce i wynosiła średnio 1294 ml

i 3,11 g/dobę, odpowiednio. Przy żywieniu dietą HP objętość soku była większa ( $P<0,01$ ) niż przy skarmianiu diet MP i LP, a ilość wydzielonego azotu przy diecie HP wynosiła 3,35 g/dobę i była większa ( $P<0,05$ ) niż przy skarmianiu diet LP i MP (2,66 g/dobę). Ogólna aktywność trypsyny i chymotrypsyny nie zależała od poziomu włókna w dawce. Poziom białka w dawce nie wpłynęła na aktywność trypsyny, natomiast aktywność chymotrypsyny była większa ( $P<0,01$ ) przy żywieniu dietą HP niż LP i MP. Skład aminokwasowy soku trzustkowego z żółcią był zbliżony przy skarmianiu wszystkich diet.

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## STRESZCZENIE

Wpływ poziomu białka i włókna w diecie na aktywność żółciowo-trzustkową u owiec

Przebadano dwa doświadczenia, każde na 6 trybach o masie ciała około 40 kg z karmieniem mieszczonym w przewodzie wójlinowym-żółciowym i prostą karmią w dwumiejscowym. Zwiększono w układzie karmienia żółciowego w doświadczeniu I - dietami składowymi: mi sie z kiana żółciowego i oleszanki (niezawijanej) 134 (LP), 200 (MP) i 311 (HP) g/kg włókna surowego, z doświadczenia II - dietami zawierającymi 130 (LP), 170 (MP) i 200 (HP) g/kg białka ogólnego przy zbliżonej zawadzie włókna. Objętość soku trzustkowego z żółcią i zawartość w nim azotu ogólnego nie zależała od poziomu włókna w dawce i wynosiła średnio 1594 ml