Journal of Animal and Feed Sciences, 10, 2001, 435 – 446

Effect of soyabean meal, rapeseed meal and extruded field bean seeds on duodenal amino acids supply in sheep

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(Received 3 August 2000; revised version 6 April 2001; accepted 7 August 2001)

ABSTRACT

In the first of two experiments, four rumen cannulated wethers were used to determine the extent of rumen protein degradation in soyabean meal (SBM), rapeseed meal (RSM) and extruded field bean seeds (EFB), using the *in sacco* method. Rumen non-degradable N in SBM, RSM and EFB of total-N was 38, 29 and 48%, respectively. In the second experiment, four rams with cannulas in the duodenum were fed isoenergetic total mixed rations (TMR) containing similar amounts of protein truly digestible in the small intestine (PDI). TMR consisted of meadow hay and concentrate (50:50, DM basis) in which SBM, SBM+RSM and SBM+EFB were the main sources of protein.

The highest, statistically significant ($P<0.05$) methionine and cystine concentrations in the duodenal digesta were found in sheep fed SBM+RSM diet, while the lowest – in those fed SBM+EFB diet. Significantly higher ($P<0.05$) flows of total N (33.49 g/day), non-ammonia N (33.08 g/day) and total amino acid (197.7 g/d) were observed in sheep fed rations with SBM + EFB.

KEY WORDS: sheep, protein sources, duodenum, amino acids

INTRODUCTION

The amino acids (AA) flowing into the small intestine of ruminants constitute a sum of three protein fractions - microbial protein synthesized in the rumen, feed protein undegraded in this forestomach and endogenous protein from digestive secretions and cell turn-over. The degree to which AA requirements of ruminants are fulfilled will depend primarily on the amount of dietary protein, its source and the extent of its degradation in the rumen (Titgemeyer et al., 1989; Benchaar et al., 1994).

The profile of AA reaching ruminants' small intestine is an important determinant of milk and wool production as well as productivity of growing ruminants (Lynch et al., 1991; Urbaniak, 1995b; Rulquin et al., 1998). However, many research papers reported that AA profile of protein absorbable from the small intestine may not always be optimal for maximal performance of different kinds of ruminant production - milk, growth and wool (Schwab 1994; Metcalf et al., 1996; Rulquin et al., 1998). Several reports indicated that AA pattern may vary considerably depending on the type of diet and source of protein (Titgemeyer et al., 1989; Clark et al., 1992; Urbaniak, 1995a; Henson et al., 1997; Rulquin et al., 1998).

Therefore, the aim of this research was to determine the effect of soyabean meal (SBM), rapeseed meal (RSM) or extruded field bean seeds (EFB) on AA profile and AA flow in the small intestine of sheep fed a hay-based diet.

MATERIAL AND METHODS

Experiment 1

Four 8-month old wethers of Polish Merino breed weighing 40 kg (SE±4 kg) fitted with a rumen cannula were used to determine the extent of rumen degradation of SBM, RSM and EFB protein according to the *in sacco* method given by Mehrez and Ørskov (1977). Samples of each feed were incubated in the rumen for 6, 12, 18 and 24 h. The field bean seeds were processed in a twin-screw extruder (Insta Pro 2000R, UK) at an exit temperature of 160°C. Commercial SBM and RSM were used in this study. The experiment was not processed statistically.

Experiment 2

Four 8-month old Polish Merino rams weighing 40 kg (SE±4 kg) fitted with a re-entrant cannula proximal to the pancreatic-bile duct were used as experimental animals. The experiment was carried out using a transposition design which comprised three consecutive periods in which animals were fed isoenergetic total mixed rations (TMR) containing SBM, SBM+RSM and SBM+EFB as main sources of protein, respectively.

Experimental animals were fed according to the INRA system (IZ-INRA, 1993). The nutritive value of rations were set up using INWAR ver. 1.0 and INRAtion ver. 2.6. (1994) software systems. All TMRs contained similar amounts of net energy

(UFL) and protein truly digestible in the small intestine (PDI). TMR consisted of concentrate and meadow hay (50:50, DM basis) which was chopped into 2 cm pieces, mixed with the concentrate every day morning and in this form offered to animals. The composition of concentrates, TMR chemical composition and nutritive value are given in Tables 1, 2 and 3.

Throughout the experiment the rams were housed in individual cages. The diets of 1180 g per head/day (DM basis) were split into two equal parts and fed to animals at 07:00 and 19:00 h. Water was supplied to animals at each feeding and the barn was lit 24 h.

Each experimental period consisted of a 10-day initial phase followed by a 24-h collection period. The duodenal digesta was collected quantitatively, meas-

TABLE 1

Composition of the concentrate mixtures, %

Component	Supplement		
	SBM	SBM+RSM	SBM+EFB
Soyabean meal	31.16	20.09	20.00
Rapeseed meal	-	20.09	-
Extruded field bean seeds	-	-	20.00
Wheat	32.34	40.08	51.00
Wheat brans	31.43	15.07	5.10
Rape seed oil	2.56	2.56	1.00
Limestone	1.60	1.21	2.00
Mineral-vitamin premix[1]	0.90	0.90	0.90

[1] contains in 1 kg: vit. A 1600000 IU, vit. D_3 130 000 IU, vit. E 2000 mg, vit. B_1 500 mg, Fe 2000 mg, Cu 1600 mg, Mn 8000 mg, Zn 9000 mg, I 80 mg, Co 20 mg, Se 30 mg, Mg 40 mg

TABLE 2

Chemical composition of total mixed rations (TMR), g/ kg dry matter

Item	Supplement		
	SBM	SBM+RSM	SBM+EFB
Dry matter	884.1	902.2	910.0
Crude protein	158.3	186.3	140.4
Ether extract	30.0	28.6	27.4
Crude fibre	159.5	191.9	232.6
N-free extractives	573.1	512.2	524.9
Crude ash	79.1	81.0	74.7
Acid detergent fibre	212.9	228.1	262.5
Neutral detergent fibre	454.0	443.7	491.8

TABLE 3

Nutritive value of experimental total mixed rations (TMR)

Item	Supplement		
	SBM	SBM+RSM	SBM+EFB
In 1 kg of DM, INRA system:			
UFL	0.91	0.93	0.92
PDI, g			
PDIN	120	119	114
PDIE	110	109	105

IZ-INRA (1993): UFL – net energy, unit for milk production; PDI – protein truly digestible in the small intestine: PDIN – PDI dependent on fermentable N amount: PDIE – PDI dependent on ruminally available energy amount

ured every three hours, heated to the temperature of 38°C and infused to the distal part of the duodenum. Aliquots constituting 5% of the collected digesta from each animal were then composited and a sub-sample frozen for subsequent analysis of NH_3-N. The remainder of the duodenal composite was lyophilized and ground (2- mm screen) for further analyses.

Standard methods were used to determine the chemical composition of feeds and duodenal digesta (AOAC, 1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined by the method of Van Soest et al. (1991).The content of NH_3-N in the duodenal digesta was analysed using Conway method (Conway, 1962). Dry matter of the small intestine digesta was calculated from the difference between the weights of samples before and after lyophilization, while non-ammonia nitrogen (NAN) from the difference between the amount of total-N and NH_3-N. Amino acid composition of feeds and lyophilizates of duodenal digesta were determined using an automatic amino acid analyser (T-339, Mikrotechna, Czech Republic), after hydrolysis with 6N HCL (24 h, 105°C). Methionine and cystine were analysed in samples prepared according the method given by Urbaniak (1995a).

Results were analysed using one-way analysis of variance, with multiple interval test according to Statgraphics Plus 6.0 (1992) software, used to compare treatment means with significant F values.

RESULTS

Experiment 1

The ruminal escape N values determined in this experiment for SBM, RSM and EFB were 38, 29 and 48%, respectively.

Experiment 2

The significant ($P<0.05$) influence of dietary supplements on duodenal flow of different N fractions was observed (Table 4). The animals from individual groups consumed different quantities of N, which resulted from both - different crude protein content and differences in the intake of individual feeds. The highest duodenal N flow was recorded in animals fed SBM + EFB (33.49 g/d) and the lowest - in rams given SBM diets (30.94 g/d). The recovery of total-N in the duodenum exceeded N intake in all experimental diets. The lowest NH_3-N flow through the duodenum was found in sheep fed the diet containing SBM+EFB (0.41 g/d) and the highest in SBM + RSM (0.77 g/d). The duodenal flow of NAN was found to differ among treatments. The highest flow of this N fraction was determined in animals fed SBM + EHB (33.08 g/d) and the lowest in SBM diet (25.80 g/d). Rams fed SBM + RSM diets reached intermediate values.

TABLE 4

N intake and flow through duodenum of total-N, ammonia-N (NH_3-N) and non- ammonia-N (NAN)

Item	Supplement			SE[1]
	SBM	SBM+RSM	SBM+EFB	
N intake, g/day	20.14[a]	24.88[b]	24.13[b]	0.73
Duodenal flow				
total N, g/day	26.49[a]	30.94[b]	33.49[b]	1.80
total N, % intake	131.53[a]	124.40[b]	138.79[c]	4.28
NH_3-N, g/day	0.69[a]	0.77[a]	0.41[b]	0.02
NAN, g/day	25.80[a]	30.17[b]	33.08[b]	1.29

[1] standard error

[a,b,c] means in the same row followed by different letters differ at $P<0.05$

Dietary protein AA composition (Table 5) expressed as the sum of essential AA (EAA), non-essential AA (NEAA) and total AA (TAA) was similar. However, differences in the content of individual AA were detected. The highest amounts of methionine and cystine (1.8 and 1.4 g/16 gN, respectively) were found in SBM+RSM diets and the lowest in SBM+ EFB rations (0.8 and 0.9 g /16 g N, respectively). The content of lysine in the protein of all diets was similar.

AA profile of protein absorbable from the small intestine (Table 6) differed from that of the dietary protein AA. The source of protein in TMR influenced the content in duodenum of some EAA - methionine, cystine and histidine as well as tyrosine and glycine. The sum of EAA, NEAA and TAA did not significantly ($P>0.05$) differ among treatments.

TABLE 5

Amino acid composition of diets, g/16 g N

Amino acid	Supplement		
	SBM	SBM+RSM	SBM +EFB
Essential amino acids			
Lys	5.2	5.2	5.1
His	2.8	3.5	2.8
Thr	3.6	3.7	3.9
Arg	5.8	3.5	5.9
Val	5.0	5.1	5.2
Met	1.1	1.8	0.8
Cys	0.9	1.4	0.9
Ile	4.0	4.0	4.0
Leu	7.0	8.0	7.2
Phe	5.5	5.6	5.8
Non-essential amino acids			
Tyr	3.6	4.1	4.1
Asp	10.0	9.0	9.4
Glu	19.7	19.5	17.8
Ser	4.3	4.3	4.3
Gly	4.2	4.4	4.6
Ala	5.3	4.9	5.6
Pro	7.7	7.7	7.5
Total EAA	40.9	41.8	41.6
Total NEAA	54.8	53.9	53.3
Total amino acids	95.7	95.7	94.9

Animals from all groups were fed diets containing a similar amount of PDI, but different amounts of crude protein. As feed intake was also different, this caused significant ($P<0.05$) differences in AA intake (Table 7). The highest intakes of EAA, NEAA and TAA were observed in animals fed SBM + RSM and the lowest in the group fed SBM. However, the highest ($P<0.05$) flow of EAA, NEAA, TAA through duodenum (Table 8) was noted in sheep fed SBM+EFB, on the other hand the flow of methionine and cystine was lower in the case of this group.

DISCUSSION

The performed study characterized the effect of SBM, RSM and EFB on the AA profile and the AA flow in the sheep duodenum. Individual protein sources

TABLE 6

Amino acid composition of duodenal digesta, g/16 g N

Amino acid	Supplement			SE
	SBM	SBM+RSM	SBM +EFB	
Essential amino acids				
Lys	6.2	6.1	6.2	0.3
His	2.7[a]	2.6[a]	1.9[b]	0.3
Thr	5.1	5.0	4.9	0.2
Arg	4.5	3.9	3.8	0.3
Val	5.6	5.4	5.2	0.3
Met	1.4[a]	1.9[b]	1.2[a]	0.1
Cys	1.5[a]	1.7[a]	1.2[b]	0.1
Ile	4.2	4.3	4.4	0.3
Leu	7.4	7.5	7.1	0.3
Phe	5.6	5.2	5.5	0.2
Non-essential amino acids				
Tyr	4.0[a]	4.5[a]	5.7[b]	0.2
Asp	10.6	10.7	10.3	0.1
Glu	14.4	14.2	13.7	0.3
Ser	5.4	5.5	5.1	0.1
Gly	5.7[a]	5.4	7.6	0.2
Ala	6.2	6.1	6.4	0.2
Pro				
Total EAA	42.4	43.6	41.4	0.6
Total NEAA	50.7	51.3	53.1	0.7
Total amino acids	93.1	94.9	94.5	0.6

[a,b] means in the same row followed by different letters differ at $P<0.05$

differed in the extent of ruminal degradation. The degradability of SBM and RSM crude protein found *in sacco* method in these investigations were comparably with those reported elsewhere in the literature (Madsen and Hvelplund, 1985; Masoero et al., 1994). The value for EFB ruminal escape N (48%) observed in performed experiment was a little higher than in study conducted by Benchaar et al. (1994) and the differences can be attributed to different technological parameters of extrusion.

Total mixed rations applied in the study contained different protein sources. The diets contained similar amounts of PDI, however, the content of crude protein varied and amounted to 158, 186 and 140 g in 1 kg DM in SBM, SBM+RSM and SBM+EFB rations, respectively. These differences and the fact that individual animals consumed varying amounts of feed caused significant differences in total-N intake.

TABLE 7

Amino acid intake from rations, g/day

Amino acid	Supplement			SE
	SBM	SBM+RSM	SBM +EFB	
Essential amino acids				
Lys	6.5^{Aa}	8.1^{Bb}	7.7^{Bc}	0.2
His	3.5^{Aa}	5.4^{Bb}	4.2^{Ac}	0.1
Thr	4.5^{a}	5.7^{b}	5.9^{bc}	0.2
Arg	7.3^{Aa}	5.4^{Bb}	8.9^{Ac}	0.2
Val	6.3^{a}	7.9^{a}	7.8^{b}	0.2
Met	1.4^{Aa}	2.8^{Bb}	1.2^{Aa}	0.1
Cys	1.1^{Aa}	2.2^{Bb}	1.3^{Aa}	0.1
Ile	5.0^{a}	6.2^{b}	6.0^{b}	0.2
Leu	8.8^{Aa}	12.4^{Bb}	10.8^{Bc}	0.7
Phe	6.9^{a}	8.7^{b}	8.7^{b}	0.3
Non-essential amino acids				
Tyr	4.5^{Aa}	6.4^{Bb}	6.2^{Bb}	0.2
Asp	12.6^{a}	14.0^{b}	14.2^{b}	0.8
Glu	24.8^{Aa}	30.3^{Bb}	26.8^{Aa}	1.2
Ser	5.4^{a}	6.7^{b}	6.5^{b}	0.3
Gly	5.3^{a}	6.8^{b}	6.9^{b}	0.2
Ala	6.7^{a}	7.6^{ab}	8.4^{b}	0.4
Pro	9.7^{Aa}	12.0^{Bb}	11.3^{Ab}	0.5
Total EAA	51.3^{a}	64.8^{b}	62.5^{b}	2.1
Total NEAA	69.0^{a}	83.8^{b}	80.3^{b}	3.0
Total amino acids	120.3^{Aa}	148.6^{Bb}	142.8^{Bb}	5.8

$^{A, B; C; a, b, c}$ means in the same row followed by different letters differ: big letters $P<0.01$, small letters $P<0.05$

In all treatments the duodenal flow of total-N was higher than N intake. The highest value of this parameter was observed in animals fed SBM+EFB and the lowest in sheep obtained SBM+RSM diets. The phenomenon of N recoveries greater than 100% has been reported elsewhere. Loerch et al. (1983) reported increases in N flow to the duodenum of 18, 27, 58, 59 and 81% for diets supplemented with urea, meat meal, soyabean meal, blood meal and dehydrated lucerne, respectively. Similarly, Price et al. (1988) observed that in cows fed dehydrated lucerne, total-N duodenal flow was 11% higher than intake. In the experiment conducted by Urbaniak (1995a) on sheep, total-N recovery in the duodenum exceeded N-intake in diets with fish meal, blood meal, and soyabean meal (133, 121 and 114%, respectively), but with casein - it was slightly lower (97%).

TABLE 8

Amino acid flow through sheep duodenum, g/day

Amino acid	Supplement			SE
	SBM	SBM+RSM	SBM +EFB	
Essential amino acids				
Lys	10.0^{a}	11.8^{bc}	13.0^{c}	0.3
His	4.3	5.0	4.0	0.1
Thr	8.2	9.7	10.2	0.2
Arg	6.9	7.5	7.9	0.2
Val	9.0	10.4	10.9	0.3
Met	2.3^{a}	3.7^{b}	2.5^{a}	0.1
Cys	2.4^{a}	3.3^{b}	2.5^{a}	0.2
Ile	6.8^{a}	8.3^{b}	9.2^{b}	0.3
Leu	11.9	14.5	14.9	0.3
Phe	9.0	10.0	11.5	0.4
Non- essential amino acids				
Tyr	6.4^{a}	8.7^{b}	11.9^{c}	0.2
Asp	17.1	20.7	21.5	0.1
Glu	23.2^{a}	27.5^{b}	28.7^{b}	0.3
Ser	8.7	10.6	10.7	0.2
Gly	9.2^{a}	10.4^{a}	15.9^{b}	0.2
Ala	10.0^{a}	11.8^{a}	13.4^{b}	0.2
Pro	7.1	9.5	9.0	0.2
Total EAA	70.8^{a}	84.2^{b}	86.6^{b}	2.8
Total NEAA	81.7^{a}	99.2^{bc}	111.1^{c}	3.4
Total amino acids	152.5^{a}	183.4^{bc}	197.7^{c}	5.9

$^{a, b, c}$ means in the same row followed by different letters differ at P<0.05

Duodenal NAN flow exceeded daily N intake in all treatments. Probably it was a result of microbial synthesis in the rumen and recycled endogenous N and, in the case of SBM+EFB diet, also of a lower extent of ruminal degradability of extruded field bean seeds.

AA profile of protein absorbable in small intestine as well as quantities of individual AA flowing through this part of the gastrointestinal tract are among the most important factors affecting ruminant productivity (Rulquin et al., 1993; Mebjeesh et al., 1996). Researchers are particularly interested in the content, in the protein of small intestine digesta, of two EAA, such as methionine and lysine, which can affect the biological value of dietary protein and can be limiting for lactating dairy cows and goats (Fraser et al., 1991; Bequette et al., 1998; Rulquin et al., 1998). In the case of sheep, sulphur AA (S-AA) – methionine and cystine are especially important as they can limit milk yield and its composition, wool production and animal growth (Pisulewski and Buttery, 1985; Cottle, 1988; Urbaniak et al., 1998).

The AA profile of duodenal digesta protein is primarily the resultant of the AA composition of microbial protein and dietary protein undegraded in the rumen.

In the study discussed here the content of lysine in the protein of all diets was similar. The amount of this AA was also almost identical in the protein of duodenal digesta of all experimental animals, although its share in the total AA was higher. On the other hand, the content of methionine and cystine varied markedly both in dietary protein as well as in the protein found in the small intestinal digesta. The highest content of these AA in the protein of the SBM+RSM diet (3.2%) and the lowest in the SBM+EFB diet (1.7%) was associated with the proportion of RSM or EFB in diets. These additives are respectively, either rich or poor in methionine and cystine (Benchaar et al., 1994; Moshtaghi Nia and Ingals, 1995). The content of S-AA in the protein reaching the duodenum increased markedly in all cases, especially, SBM and SBM+EFB diets (2.9 and 2.4% of crude protein, respectively). This can probably be attributed to the synthesis of microbial protein which is a relatively rich source of methionine and cystine (Santos et al., 1998). Nevertheless, protein of the duodenal digesta of sheep fed the SBM+EFB diet contained the lowest quantity of absorbable S-AA.

Results of the duodenal AA flow are difficult to interpret mainly because of differences in the intake of individual diets. Nonetheless, the highest daily flow of the sum of EAA, NEAA and TAA found in animals fed the SBM+EFB diet, at a simultaneous lower intake of feed in relation to the SBM+RSM diet, points to a higher effectiveness of EFB in the supply of the total AA to the small intestine. At the same time, the flow of S-AA shows clearly that the addition of EFB in conditions of this experiment was a factor limiting the supply of methionine and cystine to the lower gastrointestinal tract of the experimental animals.

CONCLUSIONS

It can be stated that the source of protein in the ration and the extent of its rumen degradation can affect both the amount and the profile of AA entering the small intestine of sheep. Extruded field bean seeds, which are characterised by a relatively low sulphur AA content and also by a relatively low extent of rumen degradability, can be a factor limiting the supply of methionine and cystine in the protein absorbable in the small intestine.

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STRESZCZENIE

Wpływ śruty sojowej, rzepakowej i ekstrudowanych nasion bobiku na przepływ aminokwasów w dwunastnicy owiec

Przeprowadzono dwa doświadczenia, pierwsze na 4 skopach z kaniulami żwaczowymi, w którym określano stopień rozkładu w żwaczu białka poekstrakcyjnych śrut: sojowej (SBM) i rzepakowej (RSM) oraz ekstrudowanych nasion bobiku (EFB). Udział N nie ulegającego rozkładowi w żwaczu w N-ogólnym wynosił odpowiednio 38, 29 i 48%.

Drugie doświadczenie wykonano na 4 trykach z kaniulami do dwunastnicy, żywionymi izoenergetycznymi dawkami, składającymi się z siana łąkowego i paszy treściwej (50:50), w których główne źródło białka stanowiły odpowiednio SBM, SBM+RSM i SBM+EFB. Największą, statystycznie istotną (P<0,05), zawartość metioniny i cystyny w treści dwunastniczej stwierdzono u owiec żywionych dawką zawierającą SBM+RSM, a najmniejszą u zwierząt otrzymujących SBM+EFB. Największa ilość (P<0,05) N-ogólnego (33,49 g/d), N-nieamonowego (33,08 g/d), oraz ogólnej sumy aminokwasów (197,7 g/d) przepływała u owiec otrzymujących dawkę SBM+EFB.