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Effect of acetylated soyabean peptides on rumen fermentation and nitrogen metabolism in sheep

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ABSTRACT

The objective of this study was to determine the effect of acetylated peptides on rumen fermentation and nitrogen metabolism in sheep. Six adult Poll × Dorset crossbred sheep, fitted with permanent rumen and duodenal fistulas, were used in a replicated 3 × 3 Latin design experiment. Three basic diets, balanced to similar nitrogen intake, were supplemented with 100 g soyabean meal (SBM), 60 g soyabean peptides (SBP) or 80 g acetylated soyabean peptides (ASP), respectively. The crude protein of soyabean peptides and acetylated peptides powder were 66.8 and 51.0%. The degree of acetylation was 88.9%. Soyabean peptides had the highest rumen pH (6.94), followed by ASP and SBM (6.74 and 6.58; $P<0.05$). Ruminal ammonia was also affected by treatment (8.05 and 10.18 mg/dl for ASP and SBP, respectively; $P<0.05$). Blood urea nitrogen of the SBM diet showed a higher value compared with SBP and ASP (5.96, 4.14 and 2.90 mmol/l; $P<0.05$). Apparent nitrogen digestibility of ASP (73.51%) was significantly higher than that of SBM (62.85%; $P<0.05$).

KEY WORDS: acetylated peptides, nitrogen metabolism, sheep

INTRODUCTION

Protected protein and amino acids are potentially important sources of amino acids for protein synthesis in ruminants, as they avoid the wasteful metabolism of these nutrients by rumen microorganisms and they can correct the imbalanced composition of amino acids reaching the small intestine (Leng and Nolan, 1984). Small peptides are protected very effectively from degradation in rumen fluid *in vitro* when they had been treated with various anhydrides. It is therefore proposed that N-terminal modification of peptides might be a means of supplying rumen non-degradable amino acids to

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ruminants, or protecting other rapidly degraded peptides present in feeds (Wallace, 1992a; Wallace et al., 1993). Witt et al. (1998) reported that the rumen microbial population did not adapt to utilize acetylated casein peptides. But there have been few studies to evaluate the effect of protected soyabean peptides on rumen fermentation, blood urea nitrogen (BUN) and nitrogen metabolism. The aim of this study was to investigate rumen fermentation characteristics and the nitrogen metabolism of acetylated peptides compared with soyabean protein and untreated peptides.

MATERIAL AND METHODS

Acetylation of peptides

A pancreatic soya protein hydrolysate was used either as soyabean peptides or acetylated peptides. Soyabean peptides with a degree of hydrolysis (DH) of about 25% were produced by treatment with Alcalase and Neutrase (T=50°C, pH=10, reaction time=5.5 h), and were collected by ultrafiltration membranes. The molecular weight cutoff was less than 3000 Da. Acetylated peptides were prepared by a method used previously (Wallace, 1992a), with some modifications. The results showed that the optimal conditions of acetylation was a temperature <10°C, peptides concentrations=5% (W/V, based on CP), pH=8.5, ratio of acetic anhydride to peptides=40% (V/W), reaction time=2 h, followed by spray-drying the liquid. The degree of acetylation was determined by reaction with ninhydrin (Moore and Stein, 1954). The molecular weight distribution of the peptides was determined by gel filtration (Wallace, 1992b).

Animals and diets

Six adult Poll × Dorset crossbred sheep, fitted with permanent rumen and duodenal fistulas, were used in a replicated 3 × 3 Latin square design experiment. Sheep were fed with a basal diet of hay, maize, sodium bicarbonate, salt and a vitamins-minerals mix (700, 280, 6, 8 and 6 g kg^{-1} of DM, respectively), and tested against three treatments consisting of the basal diet supplemented with 100 g soyabean meal (SBM), 60 g soyabean peptides (SBP) or 80 g acetylated soyabean peptide (ASP), respectively (based on identical CP level). Meals were given twice daily at 08.00 and 18.00 h. Experimental periods were 15 d in duration (10 d of treatment adaptation and 5 d of data collection).

Ruminal pH, ammonia and blood urea nitrogen

Ruminal fluid samples (50 ml) were taken with a rumen filter probe tube *via* the rumen cannula at 0, 1, 2, 3, 4, 6 and 8 h after the morning feeding on day 11. Samples

were filtered through four layers of cheesecloth. Rumen liquid pH was immediately determined using a hand held pH electrode (Model pHB-4, Shanghai Chemical Co. China). Additionally, 10 ml of filtered rumen fluid was centrifuged at 4000 *g* for 30 min at 4°C to obtain a clear supernatant, which was analysed for ammonia using a phenol-hypochlorite assay (Broderick and Kang, 1980). At the end of each experimental period (at 10.30 h of d 15), blood samples were picked from the jugular vein of each sheep. Blood samples were collected in tubes with sodium heparin for BUN analysis (Blood Urea Nitrogen Kit, C013, Spectrophotometry, China).

Digestibility

Digestibility of nitrogen was determined by daily collection of total faeces and urine from d 12 to d 14 (72 h). All faeces and urine were weighed at 07.30 and 19.30 h each day, and a faeces sample (5% fresh weight) was collected after thorough mixing. A urine sample (5% of urine) was collected for nitrogen estimation.

Experimental measures and sample analysis

During sampling periods (from d 11 to d 15), feed and orts from each sheep were gathered daily for calculation of DM intake. Dry matter of feed and faeces were determined by oven-drying at 105°C for 16 h, OM was determined by ashing at 550°C for 8 h, and CP was determined by the micro-Kjeldahl method (AOAC, 1990).

Statistical analysis

Data on all variables were analysed using the PROC MIXED model procedure (SAS, 1998). Significance was declared at $P \leq 0.05$. A trend was considered to exist if $0.05 < P \leq 0.10$. All reported values are least square means unless otherwise stated.

RESULTS

The gel filtration profile (data not shown) of the peptides preparation used in the present experiment showed that peptides had an average molecular mass of 1500 Da. The average peptide chain length of SBP and ASP was 4.0. The crude protein of soyabean peptides and acetylated peptides powder were 66.8 and 51.0%, respectively. The acetylation degree was 88.9% as determined using ninhydrin.

Ruminal pH, ammonia and BUN are shown on Table 1. Soyabean peptides had the highest pH (6.94), followed by ASP and SBM (6.74 and 6.58; $P<0.05$). Ruminal ammonia was affected by treatment (8.05 and 10.18 mg/dl for ASP and SBP; respectively; $P<0.05$). Blood urea nitrogen of SBM had the highest value compared with SBP and ASP (5.96, 4.14 and 2.90 mmol/l; $P<0.05$).

Table 1. Effects of soyabean meal, soyabean peptides and acetylated peptides on ruminal pH, ammonia and BUN[1]

	SBM[1]	SBP[1]	ASP[1]
pH	6.58 ± 0.17[a2]	6.94 ± 0.17[b]	6.74 ± 0.10[a]
NH3-N, mg/dl	14.98 ± 5.16[a]	10.18 ± 4.57[a]	8.05 ± 6.51[b]
BUN, mmol/l	5.96 ± 0.65[a]	4.14 ± 0.53[b]	2.90 ± 0.44[c]

[1] SBM - soyabean meal, SBP - soyabean peptides, ASP - acetylated peptides, BUN - blood urea nitrogen
[2] means within a row with different superscripts differ (P<0.05)

At a similar nitrogen intake, nitrogen losses from the faeces of ASP were significantly less than that of SBM (P<0.05) (Table 2). Similarly, nitrogen losses from the urine were higher for SBM than for SBP and ASP (P<0.05). Retained nitrogen, and the ratio of nitrogen retention and digested nitrogen were significantly higher for ASP and SBP than for SBM (P<0.05). Apparent nitrogen digestibility of ASP was significantly higher than those of SBM and SBP (P<0.05).

Table 2. Effects of soyabean meal, soyabean peptides and acetylated peptides on nitrogen retention and nitrogen digestion

Indices	SBM[1]	SBP[1]	ASP[1]
Intake nitrogen, g/d	17.43 ± 0.42[a2]	17.18 ± 0.08[a]	17.15 ± 0.14[a]
Faeces nitrogen, g/d	6.44 ± 0.74[a]	5.06 ± 0.41[ab]	4.54 ± 1.16[b]
Urinary nitrogen, g/d	7.68 ± 1.12[a]	5.19 ± 1.23[b]	5.01 ± 1.30[b]
Digested nitrogen, g/d	10.90 ± 0.64[a]	12.12 ± 0.23[b]	12.60 ± 1.40[b]
Nitrogen retained[2], g/d	3.60 ± 0.86[a]	6.93 ± 1.29[b]	7.76 ± 3.26[b]
Apparent biological value of dietary N, %	20.83 ± 5.09[a]	40.35 ± 5.09[b]	45.24 ± 9.36[b]
Nitrogen retention: digested N, %	33.02 ± 0.01[a]	57.20 ± 0.01[b]	61.55 ± 0.01[b]
Apparent nitrogen digestibility, %	62.85 ± 3.81[a]	70.53 ± 2.23[a]	73.51 ± 3.07[b]

[1] SBM - soyabean meal, SBP - soyabean peptides, ASP - acetylated peptides, [2] nitrogen retained - intake nitrogen - faeces nitrogen - urinary nitrogen
[2] means within a row with different superscripts differ (P<0.05)

DISCUSSION

N-terminal modification was shown to result in protection of peptides from degradation by rumen microorganisms in *in vitro* experiments, especially for peptides of low molecular weight (Wallace, 1992a; Wallace et al., 1993). In the present experiment, the concentrations of ammonia a product of amino acid breakdown, were lower with the ASP than with SBM and SBP diets. Ammonia *in vivo* indicates that ASP remains less degradable than SBM and SBP.

Blood urine nitrogen (BUN) is the major end product of nitrogen metabolism in ruminants, and high concentrations of BUN are indicative of an inefficient utilization

of dietary nitrogen. Based on the result of this study, ASP had the lowest value, followed by SBP and SBM. This difference was likely attributable to variation in dietary protein content with a lower protein degradation in ASP diet.

Acetylated peptides had a high digestibility in the sheep intestines. The result was similar with that of Wallace et al. (1998), who injected acetylated peptides into the duodenum of sheep. It is clear that these *in vivo* studies suggest a benefit from acetylation in controlling ruminal breakdown and providing nitrogen to ruminants. In this experiment, nitrogen retention *in vivo* indicated that sheep obtained more nitrogen accretion when fed ASP compared with SBM. Moreover, it is believed that most of the acetylated peptides have been deacylated, and turned to peptides in the abomasum before they reach the intestine because of the low pH in the abomasum. The results showed that the increased nitrogen retained was a consequence of acetylated peptides being degraded more slowly.

CONCLUSIONS

Based on the results of this study, acetylated peptides had the power to resist the activity of ruminal peptidase. Ruminants might benefit from acetylated peptides supplementation because of low degradability in the rumen and high digestibility in the intestine.

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