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Effects of 2-hydroxy-4(methylthio) butanoic acid isopropyl ester on rumen fermentation in cashmere goats[1]

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ABSTRACT - The impact of dietary 2-hydroxy-4-(methylthio) butyric acid isopropyl ester (HMBi) on rumen fermentation in cashmere goats was investigated to determine the effect of this ester on cashmere production. Four healthy castrated Liaoning cashmere goats with permanent rumen cannulas were assayed for four periods. In each period, goats were fed a different diet: control diet (containing 0% MetaSmart (an HMBi product)), diet I (containing 0.85% MetaSmart), diet II (containing 1.27% MetaSmart) or diet III (containing 1.70% MetaSmart). Rumen pH, ammonia-N concentration, volatile fatty acid (VFA) concentrations and total protein concentration were determined after feeding in each period. Results showed that rumen pH significantly increased and rumen ammonia-N concentration significantly decreased when the diet was supplemented with 0.85%, 1.27% and 1.70% MetaSmart. Diets with 1.27% and 1.70% MetaSmart increased rumen total VFA concentration, and 3 hours post feeding all three HMBi diets increased acetic acid concentration. Rumen total protein concentration significantly increased in the goats fed 1.27% or 1.70% MetaSmart. Thus, diets supplemented with HMBi significantly affected rumen pH, ammonia-N concentration, VFA concentrations and total protein content. Dietary HMBi can promote rumen fermentation in cashmere goats.

Key Words: 2-hydroxy-4-(methylthio) butyric acid isopropyl ester, rumen ammonia-N, rumen pH, rumen total protein, rumen volatile fatty acids

Introduction

Methionine is a limiting amino acid in the diet of goats. As a sulfur-containing amino acid, methionine is a key factor to determine the quality of cashmere (Reis, 1979) and consequently there has been much interest in increasing methionine levels in the diet of cashmere goats.

2-hydroxy-4-(methylthio) butyric acid isopropyl ester (HMBi) is a rumen-protected methionine additive that can be absorbed by the rumen wall and then converted into methionine (Graulet et al., 2004). However, about 50% of HMBi is degraded to 2-hydroxy-4-(methylthio) butyric acid (HMB) by microbes in the rumen (Robert et al., 2002; Noftsger et al., 2005), so HMBi may affect rumen fermentation via HMB. Numerous studies have investigated the effect of HMB on ruminants. Iriki et al. (1989) concluded that HMB can promote rumen fermentation in Saanen dairy goats. Patton et al. (1970) stated that HMB affected rumen fermentation by altering some microbes in the rumen, while Hegedus et al. (1995) and Noftsger et al. (2005) found that HMB did not significantly promote the growth of microbes. These varying results cast doubt about the effects of HMB on rumen fermentation and microbe populations, making it difficult to assess the effects of HMBi on rumen fermentation.

The benefits of feeding HMBi to cashmere goats need to be determined. This study was conducted to evaluate the effects of dietary HMBi supplementation on rumen fermentation in cashmere goats in order to determine the effects on cashmere production.

Material and Methods

The experimental design was a 4×4 Latin-square with four healthy 1-year-old castrated Liaoning cashmere goats weighing about 30 kg each. The four goats were placed in four equally sized pens and all the goats had permanently installed ruminal cannulas. There were four experimental periods, each of which consisted of 14 d: 7 d for adaptations and 7 d for testing. In each experimental period, goats were fed twice a day (06h00 and 18h00) with a different diet. All goats were offered feed and water *ad libitum* under the same environmental conditions.

The HMBi source was the product called MetaSmart (supplied by Adisseo company), containing ≥57% HMBi. The basal diet (control diet; Table 1) was formulated by

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reference to NRC (1985), diet I was the basal diet with 0.85% MetaSmart, diet II included 1.27% MetaSmart and diet III had 1.70% MetaSmart.

Rumen fluid samples (30 mL) were collected four times per day (once every 6 hours) from the fourth day of formal period. Sampling time was different each day during the 3 d so that it was evenly distributed into different time frames. pH was determined with a digital pH meter immediately following sample collection. The sample was then filtered with a four-layer wool fabric mesh, centrifuged at 3500 rpm for 10 min and the supernatant was mixed with 50% sulfuric acid to obtain a ratio of 50:1 (supernatant to sulfuric acid, v/v). Samples were kept at –20 ºC until ammonia-N and volatile fatty acid (VFA) analyses. Ammonia-N concentration was analyzed using the colorimetric method (Feng & Gao, 1993). Rumen VFA concentration was determined by gas chromatography after pretreatment using the method of Erwin et al. (1961). An aliquot of 0.5 mL rumen fluid was added to 9.5 mL trichloroacetic acid, centrifuged at 4000 rpm for 10 min and then 5% sodium hydroxide was added to dissolve the pellet and dilute to a volume of 25 mL. The diluted sample was centrifuged at 4000 rpm for 10 min and the total protein content of the supernatant was determined by using the kit supplied by Nanjing Jiancheng Bioengineering Co. Ltd. (Nanjing, China).

For statistical analysis, software SAS (Statistical Analysis System, version 9.2) for Windows was used. Data are presented as mean plus standard deviation. ANOVA was performed with the GLM procedure and significant differences between treatment groups were determined by Duncan's multiple range test. The NLIN procedure was used for regression analysis between variables.

Table 1 - Composition and nutritional level of the basal diet (dry matter-based)

Ingredients	Content (%)	Nutrients	Nutrient level[2]
Hay	70.00	Metabolic energy, MJ/kg	8.89
Corn	18.00	Crude protein, %	12.29
Wheat bran	4.50	Calcium, %	0.44
Cottonseed meal	3.60	Phosphorus, %	0.23
Soybean meal	1.80	Nitrogen, %	1.97
$CaHPO_4$	0.18	Sulfur, %	0.15
Urea	0.90	Methionine, %	0.21
NaCl	0.60		
MgO	0.12		
Premix[1]	0.30		
Total	100.00		

[1] Provided per kg of premix: $FeSO_4 \cdot H_2O$ - 120 g; $Cu_2(OH)_3Cl$ - 30 g; $ZnSO_4 \cdot H_2O$ - 160 g; $Ca(IO_3)_2$ - 25 g; Na_2SeO_3 - 14 g; $MnSO_4 \cdot H_2O$ - 90 g; $CoCl_2 \cdot 6H_2O$ - 1 g; vitamin A - 50 g; vitamin D3 - 10 g.
[2] The level of metabolic energy is a calculated value, while the levels of the other nutrients are measured values.

Results

Rumen pH gradually decreased after feeding with each diet (Table 2). The lowest pH, which was above 6.0, occurred about 5 hours after feeding, after which it gradually increased. The effect of dietary HMBi concentration on average rumen pH was significant within 12 hours ($P<0.05$). The pH values with diets I, II and III were significantly higher than that of the control diet ($P<0.05$), but there were no significant differences among the three diets supplemented with HMBi ($P>0.05$). Significant correlation was found between rumen pH and dietary HMBi level ($P<0.01$), with a regression equation of $Y = 6.69 + 0.11X_1 - 0.19X_2 + 1.54X_2^2$, where Y, X_1 and X_2 represent rumen pH, dietary MetaSmart levels and hours after feeding, respectively ($R^2 = 0.8598$, $n = 96$ and $P<0.01$).

Rumen ammonia-N concentration gradually increased after feeding with each diet (Table 3), but the increasing trend was greater with the control diet than with the three other diets. The highest ammonia-N concentration was achieved at about 3 hours after feeding, after which it gradually decreased. Supplementation of HMBi had a significant effect on rumen ammonia-N concentrations from 3 to 11 hours and on the average concentration within 12 hours after feeding ($P<0.05$). Ammonia-N concentration was not significantly different among diets I, II and III ($P>0.05$) but was significantly higher in the control diet ($P<0.05$). Significant correlation was verified between rumen ammonia-N concentration and dietary HMBi level ($P<0.01$), with a regression equation of $Y = -0.44 + 3.39X_1 + 12.93*\exp(-X_1) + 26.12*\exp(-0.94X_2)*X_2^{1.95}$, where Y, X_1 and X_2 represent rumen ammonia-N concentration, dietary MetaSmart levels and hours after feeding, respectively ($R^2 = 0.9592$, $n = 96$ and $P<0.01$).

After feeding with diets supplemented with HMBi, the variation in total VFA concentration was similar to that of the control diet (Table 4). The volatile fatty acid concentration gradually increased to peak values at about 3 hours after feeding, after which VFA concentration decreased. There were significant effects of dietary HMBi supplementation on total VFA concentration at 1 and 3 hours after feeding and on the average concentration within 12 hours between two adjacent feedings ($P<0.05$). Rumen total VFA concentration with the control diet was significantly lower than that of diets II and III ($P<0.05$). At 3 hours after feeding (Table 5), dietary HMBi supplementation had a significant effect on the concentration of acetic acid ($P<0.05$) but no significant effect on the proportion constituting the VFA ($P>0.05$); neither were there any significant effects on the concentrations and proportions of propionic acid and

butyric acid (P>0.05). There was no significant difference among the three diets supplemented with HMBi. There was no significant correlation between total VFA concentration in rumen and HMBi levels in diet (P>0.05). The regression equation was expressed as $Y = 30.40 + 6.54X_1 - 1.60*\exp(X_1) + 56.54*\exp(-0.78X_2)*X_2^{1.71}$, where Y, X_1 and X_2 represent total VFA concentration in rumen, dietary MetaSmart levels and hours after feeding, respectively ($R^2 = 0.9874$, n = 96 and P>0.05).

Total protein content in rumen peaked at about 1 hour after feeding and then decreased until 5 hours post feeding with each diet; it increased and decreased before subsequent feeding (Table 6). The results also showed that HMBi significantly affected (P<0.05) total protein content

Table 2 - Rumen pH after feeding diets containing different levels of HMBi

Hours after feeding	Control diet	Diet I	Diet II	Diet III
1	6.55±0.15	6.69±0.07	6.65±0.09	6.70±0.09
3	6.30±0.14	6.30±0.08	6.35±0.15	6.35±0.08
5	6.08±0.18	6.11±0.19	6.23±0.10	6.21±0.11
7	6.17±0.12	6.34±0.16	6.37±0.13	6.38±0.07
9	6.20±0.22	6.41±0.15	6.38±0.17	6.50±0.15
11	6.44±0.20	6.59±0.11	6.66±0.08	6.55±0.21
Average	6.30±0.06b	6.41±0.06a	6.44±0.05a	6.45±0.04a

HMBi - 2-hydroxy-4-(methylthio) butyric acid isopropyl ester.
Control diet - contains 0% MetaSmart (an HMBi product); diet I - 0.85% MetaSmart; diet II - 1.27% MetaSmart; diet III - 1.70% MetaSmart.
Means in the same row followed by different letters differed significantly (P<0.05).

Table 3 - Rumen ammonia-N concentration (mg/100 mL) after feeding diets containing different levels of HMBi

Hours after feeding	Control diet	Diet I	Diet II	Diet III
1	19.73±0.32	19.32±0.13	19.32±0.84	17.81±0.33
3	26.20±0.34a	21.53±1.01b	20.16±0.39b	21.15±0.60b
5	18.01±0.17a	12.94±0.16b	11.21±0.37b	12.14±1.11b
7	14.74±0.15a	10.18±0.18b	11.07±0.41b	10.40±0.59b
9	14.00±0.15a	8.18±0.14b	7.63±0.24b	8.32±0.52b
11	13.10±0.18a	6.22±0.18b	6.78±0.29b	6.93±0.15b
Average	17.63±1.39a	13.06±1.71b	12.70±1.60b	12.80±1.54b

HMBi - 2-hydroxy-4-(methylthio) butyric acid isopropyl ester.
Control diet - contains 0% MetaSmart (an HMBi product); diet I - 0.85% MetaSmart; diet II - 1.27% MetaSmart; diet III - 1.70% MetaSmart.
Means in the same row followed by different letters differed significantly (P<0.05).

Table 4 - Total VFA concentration (mmol/L) in rumen after feeding diets containing different levels of HMBi

Hours after feeding	Control diet	Diet I	Diet II	Diet III
1	53.27±3.10b	56.30±2.92ab	61.35±2.89a	60.08±2.41a
3	62.62±3.27b	67.75±3.56ab	71.36±4.86a	69.63±3.21a
5	48.08±3.07	51.03±3.20	52.42±3.12	49.64±3.26
7	38.26±2.58	36.85±2.83	39.09±2.76	38.47±3.15
9	32.69±2.72	33.56±3.18	36.69±2.95	35.19±2.06
11	29.66±2.43	32.08±2.34	34.86±2.41	31.91±2.35
Average	44.11±2.75b	46.26±2.36ab	49.30±2.91a	47.49±2.56a

HMBi - 2-hydroxy-4-(methylthio) butyric acid isopropyl ester; VFA - volatile fatty acids.
Control diet - contains 0% MetaSmart (an HMBi product); diet I - 0.85% MetaSmart; diet II - 1.27% MetaSmart; diet III - 1.70% MetaSmart.
Means in the same row followed by different letters differed significantly (P<0.05).

Table 5 - Concentration and proportion of VFA in rumen at 3 hours after feeding diets containing different levels of HMBi

Concentration of VFA (mmol/L)	Control diet	Diet I	Diet II	Diet III
Acetic acid	38.45±2.86b	42.93±2.79a	45.32±3.81a	43.94±2.96a
Propionic acid	14.53±1.52	15.26±2.03	15.51±1.18	14.92±1.33
Butyric acid	7.26±0.83	7.38±0.90	8.12±0.56	8.35±0.95
Proportion of VFA (%)				
Acetic acid	64.40±4.56	63.37±3.88	63.51±3.73	62.92±4.27
Propionic acid	23.20±2.45	22.52±2.18	21.73±2.59	21.37±2.11
Butyric acid	11.59±1.19	10.89±1.36	11.38±1.28	11.76±1.58

HMBi - 2-hydroxy-4-(methylthio) butyric acid isopropyl ester; VFA - volatile fatty acids.
Control diet - contains 0% MetaSmart (an HMBi product); diet I - 0.85% MetaSmart; diet II - 1.27% MetaSmart; diet III - 1.70% MetaSmart.
Means in the same row followed by different letters differed significantly (P<0.05).

Table 6 - Total protein concentrations (mg/mL) in rumen after feeding diets containing different levels of HMBi

Hours after feeding	Control diet	Diet I	Diet II	Diet III
1	10.81±0.37	11.03±0.35	11.15±0.43	11.78±0.39
3	8.83±0.10b	9.44±0.55ab	10.54±0.46a	10.05±0.41a
5	9.02±0.19	8.84±0.37	9.41±0.47	9.16±0.35
7	9.12±0.18	10.01±0.20	9.63±0.13	9.55±0.24
9	8.35±0.42b	9.16±0.32ab	9.93±0.29a	10.77±0.37a
11	8.39±0.22	8.65±0.28	8.80±0.25	9.33±0.49
Average	9.09±0.21b	9.52±0.27ab	9.91±0.30a	10.11±0.31a

HMBi - 2-hydroxy-4-(methylthio) butyric acid isopropyl ester.
Control diet - contains 0% MetaSmart (an HMBi product); diet I - 0.85% MetaSmart; diet II - 1.27% MetaSmart; diet III - 1.70% MetaSmart.
Means in the same row followed by different letters differed significantly (P<0.05).

at 3 and 9 hours post feeding and the average content in the rumen. The total protein content with diet I was higher, but not significantly, than that of the control diet (P>0.05). The total protein content with diets II and III was significantly higher than that of the control diet (P<0.05) and was higher, but not significantly, than that of diet I (P>0.05). There was significant correlation between total protein concentration in rumen and dietary HMBi levels (P<0.01). The obtained regression equation was expressed as $Y= 12.14 + 0.62X_1 – 1.78X_2 + 0.29X_2^2 – 1.47X_2^3$, where Y, X_1 and X_2 represent total protein concentration in rumen, dietary MetaSmart levels and hours after feeding, respectively ($R^2 = 0.8498$, n = 96 and P<0.01).

Discussion

Rumen pH is the most direct and important parameter reflecting rumen fermentation status. It is also an important factor for altering rumen microbial protein synthesis. In this study the forage to concentrate ratio of all diets was nearly 70:30 and the feed intake was the same among goats. For these reasons the pH variation was probably similar among the different diets after feeding and was consistently in its optimal range (pH 6.0 to 7.0) for rumen microbial protein synthesis (Satter & Slyter, 1974). Han et al. (1996) found that rumen pH was not affected by supplementation with rumen-protected methionine in sheep. However, this study showed that the average pH of rumen fluid increases when the diet is supplemented with HMBi. This is consistent with previous reports that organic sulfur can increase rumen pH (Wang & Jia, 1999; Peng et al., 2001). These results presented herein suggest that supplementation with HMBi can promote synthesis of microbial proteins and degradation of crude fibers in cashmere goats.

Rumen ammonia-N content is affected by rumen wall absorption and chyme emptying velocity (Allison, 1980), which is a reflection of rumen microbial decomposition of nitrogenous substances and utilization of ammonia. The present study showed that the concentration used with each diet is optimal for microbial protein synthesis, as proposed in previous studies (Owens & Bergen, 1983; Preston & Leng, 1987). The results of this study do not support those of Sun et al. (2009), who found no significant effect of rumen-protected forms of methionine on rumen pH and ammonia-N concentration in dairy cows. However, HMBi supplementation can significantly decrease rumen ammonia-N concentration, which suggests that HMBi can promote ammonia utilization in the rumen of cashmere goats. This result may be because HMBi supplementation can enhance rumen microbial growth and the microbes can reutilize degraded ammonia-N to synthesize microbial proteins (Or-Rashid et al., 2001). However, when the dietary content of HMBi reached a certain level, ammonia-N concentration did not decrease with the increasing HMBi. This suggests that the promoting role of HMBi supplementation in both growth and utilization of rumen microbes is limited.

Volatile fatty acids are important products of microbial fermentation in the rumen and their content and composition in the rumen depend on dietary composition, rumen environment and microbial population. Noftsger et al. (2003) reported that HMB supplementation did not influence production of total VFA, but the concentration of butyric acid rose and that of propionic acid fell. Patterson & Kung (1988) found that HMB could promote fiber degradation and acetic acid production during rumen fermentation and Iriki et al. (1989) found that HMB could increase the ratio of acetic acid to propionic acid. This study indicated that dietary HMBi supplementation can improve rumen VFA production in cashmere goats, confirming that increasing sulfur in the diet can improve rumen fermentation (Cong et al., 2010). In addition, rumen concentration of acetic acid concentration increased with dietary HMBi supplementation, which is presumably due to the role of HMBi in promoting microbial fermentation and carbohydrate degradation in rumen.

Rumen proteins are mainly composed of dietary protein and rumen microbial protein. In the present study, the basal

diet and the feeding frequency were identical among goats. Thus, the variation found in rumen total protein is likely caused by the changes in the microbial protein content in the rumen; the total protein reflects the synthesis of rumen microbial protein. Ammonia-N concentration directly influences microbial protein synthesis (Or-Rashid et al., 2001) and total protein content in rumen. In this study, total protein content in the rumen reached the bottom when the ammonia-N concentration peaked, probably because an increasing concentration of ammonia-N leads to a decrease in pH, which inhibits microbial activities in the rumen (Hoover & Stokes, 1991). In this study, it was demonstrated that total protein content in rumen increased with increasing dietary HMBi levels. These results suggest that proper supplementation with HMBi in the basal diet increases VFA production in the rumen, which thus benefits the synthesis of rumen microbial proteins.

Conclusions

Dietary supplementation of HMBi significantly affects rumen pH, ammonia-N concentration and volatile fatty acid concentration and total protein content in cashmere goats. Supplementation of 2-hydroxy-4-(methylthio) butyric acid isopropyl ester can, therefore, improve rumen fermentation in cashmere goats.

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