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# Promotion of cashmere growth by sulfur supplements in cashmere goats[1]

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**ABSTRACT** - This study was conducted to investigate effects of inorganic and organic sulfur supplements on cashmere growth and their differences. Thirty-six six-month-old female Liaoning cashmere goats with a body weight of approximately 25 kg and good health were randomly assigned to three treatments: control, $ZnSO_4$ and HMBi (2-hydroxy-4-(methylthio) butyric acid isopropyl ester). The three groups were fed a basal diet, a $ZnSO_4$ diet (supplemented with 0.63% $ZnSO_4 \cdot H_2O$) and an HMBi diet (supplemented with 1.27% HMBi), respectively. Blood and cashmere samples were collected at the end of the three-month experimental period. The plasma concentrations of total protein, urea nitrogen, ammonia and amino acids; the cashmere content of amino acids and sulfur contents; the cashmere growth rates; and the diameter of the cashmere fibres were determined. The results indicated that dietary supplementation with $ZnSO_4$ or HMBi can decrease the plasma urea nitrogen concentration and increase concentrations of total protein and methionine in plasma. In addition, the two types of sulfur supplements appeared to increase the methionine, cysteine and sulfur contents in cashmere fibres. Furthermore, the supplements can accelerate cashmere growth, with no significant effect on cashmere fineness. The promotion of cashmere growth probably stems from the improvement in the protein metabolic balance, sulfur retention and sulfur-containing amino acids synthesis in cashmere goats following the $ZnSO_4$ or HMBi supplementation. In general, the $ZnSO_4$ supplement promotes greater cashmere growth than the HMBi supplement under the experimental conditions.

Key Words: 2-hydroxy-4-(methylthio) butyric acid isopropyl ester, amino acid, cashmere growth rate, sulfur, $ZnSO_4$

## Introduction

The physicochemical properties of wool are determined by its sulfur content and disulphide bond structure. Sulfur accounts for 2.7% to 5.4% of wool, with finer fibres containing a higher amount of sulfur (Qi & Lupton, 1994). Sulfur is an essential macroelement for ruminants and can be obtained only from feed. Dietary supplementation with inorganic sulfur or with ruminally protected sulfur-containing amino acids (SAA) is a practical approach to meet the nutritional requirements of ruminants (Qi et al., 1994b). Many studies have indicated that sulfur supplementation promotes the synthesis of ruminal microbial protein, as well as cellulose digestion, wool growth and improves wool quality (Reis & Schinckel, 1963; Bray & Hemsley, 1969; Qi et al., 1994a). Previous studies have shown that 0.23% was the optimal level of dietary sulfur in Liaoning cashmere goats (Zhang & Cong, 2009; Cong et al., 2010). Sulfur can promote wool growth by participating in the protein synthesis of ruminal microorganisms. The sulfur in ruminal microbial protein is in the form of sulfides, which are converted from sulfate and SAA (Bray & Till, 1975). Therefore, sulfur can be supplied in the form of sulfate or SAA for cashmere goats.

$Na_2SO_4$ is a frequently used sulfate, but $ZnSO_4$ has better palatability for cashmere goats. Among SAA, methionine is considered to be a limiting amino acid for ruminants (Nimrick et al., 1970; Fenderson & Bergen, 1975; Richardson & Hatfield, 1978). Previous work showed that ruminally protected methionine (RPMet), 2-hydroxy-4-(methylthio) butyric acid isopropyl ester (HMBi), can provide a substantial quantity of methionine for Holstein dairy cows (Phipps et al., 2008). Peng et al. (2001) found that there were significant differences among the effects of dietary supplementation with different inorganic sulfur sources on nutrient digestion and metabolism in cashmere goats. However, the differences between inorganic and organic sulfur and the effects of the two sulfur sources on related indicators in plasma and cashmere fibres are not yet known. This study was conducted to investigate the impacts of sulfur supplements on cashmere growth in Liaoning cashmere goats and the basic underlying mechanism to elucidate the supplementation effects of $ZnSO_4$ and HMBi and their differences.

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## Material and Methods

Thirty-six six-month-old female Liaoning cashmere goats with a body weight of approximately 25 kg and good health were randomly assigned to three treatments, with 12 goats per treatment: control (basal diet), $ZnSO_4$ ($ZnSO_4$ diet with 0.63% $ZnSO_4 \cdot H_2O$) and HMBi (diet with 1.27% HMBi), respectively. The diets were formulated according to those proposed by the NRC (1985). They included hay, corn, wheat bran and cottonseed meal, with a concentrate-roughage ratio of 30:70, 8.89 MJ metabolic energy per kilogram and 12.29% crude protein. The sulfur content (0.24%) in the $ZnSO_4$ diet and the HMBi diet were the same. HMBi was purchased from Adisseo Life Science Co., Ltd. (Shanghai). All the goats were housed under the same environmental conditions, and they were allowed to feed freely twice a day at 06h00 and 16h00 and were offered running water *ad libitum* during the three-month experimental period from September to December.

Fasting blood samples were collected from the jugular vein in the morning at the end of the experiment. After collection, the blood samples were centrifuged at 3500 rpm at 4 °C for 10 min and plasma was collected. The plasma concentrations of total protein, urea nitrogen and ammonia were determined with the Coomassie brilliant blue method, the glyoxime method and the colorimetric method, respectively, using kits provided by Nanjing Jiancheng Bioengineering Institute. The plasma amino acid concentration was determined with an automatic analyzer for amino acids.

Cashmere samples were collected by clipping right next to the skin in a 10 ×10 cm patch area, which was dyed with black hair dye on the left mid-side at the beginning of the experiment. The undyed part of the cashmere samples was used to analyse the following indicators. The amino acid content was determined with an automatic amino acid analyser after deproteinisation of samples with sulfosalicylic acid (25 mg/mL of plasma), referring to Spackman et al. (1958). The sulfur content of the cashmere fibres was determined by the barium sulfate gravimetric method (Zhang & Zhang, 1986). The growth rate of the undyed part of the cashmere was evaluated using 100 cashmere fibres from each goat, and the length of the fibres was measured with a ruler with 0.01 cm precision. The cashmere samples were cut into 2 mm segments, and a fibre fineness analyser was used to measure the cashmere fineness of 100 fibres per goat.

The results are presented as means with standard deviation. Variance analysis was performed with a one-way ANOVA procedure in SPSS16.0 for Windows, and significant differences between the treatment groups were determined by Duncan's multiple range test.

## Results

The results of this study showed that there were significant differences in the concentrations of plasma total protein among the groups ($P<0.05$; Table 1). The concentrations of plasma total protein in the HMBi group and the $ZnSO_4$ group were all higher than those in the control group ($P<0.05$). Moreover, there were significant differences in the concentrations of plasma urea nitrogen among the groups ($P<0.01$), and the concentrations of plasma urea nitrogen in the HMBi group ($P<0.05$) and the $ZnSO_4$ group ($P<0.01$) were all lower than those in the control group. However, there were no significant differences in the concentrations of plasma ammonia among the groups ($P>0.05$). Therefore, dietary supplementation with $ZnSO_4$ or HMBi can increase the concentration of plasma total protein and decrease the concentration of plasma urea nitrogen in cashmere goats.

There were significant differences in the concentrations of methionine ($P<0.05$), tyrosine and phenylalanine ($P<0.01$) among the groups (Table 2). The concentrations of the three amino acids in the $ZnSO_4$ group were higher than those in the control group ($P<0.01$), and the concentrations of methionine ($P<0.05$) and tyrosine ($P<0.01$) in the HMBi group were also higher than those in the control group, but there was no significant difference in the concentration of phenylalanine between the HMBi group and the control group ($P>0.05$). In addition, the concentrations of tyrosine and phenylalanine in the HMBi group were lower than those in the $ZnSO_4$ group ($P<0.01$), but there was no significant difference in the concentration of methionine between the two groups ($P>0.05$). Moreover, there was no significant difference in the concentration of other amino acids in

Table 1 - Concentrations of total protein, urea nitrogen and ammonia in plasma

| Items | Treatments | | | P-value |
|---|---|---|---|---|
| | Control | $ZnSO_4$ | HMBi | |
| Total protein (g/L) | 67.91±1.57b | 72.10±3.47a | 71.56±3.38a | 0.038 |
| Urea nitrogen (mg/L) | 161.76±14.51Aa | 125.45±12.56Bb | 134.83±17.02ABb | 0.008 |
| Ammonia (mg/L) | 0.58±0.03 | 0.50±0.02 | 0.59±0.02 | 0.125 |

In the same row, values with different uppercase letters differ significantly ($P<0.01$); values with different lowercase letters differ significantly ($P<0.05$).

plasma among the groups (P>0.05). Thus, supplementation of $ZnSO_4$ or HMBi increases the plasma concentrations of methionine and tyrosine, and $ZnSO_4$ supplement also increases plasma concentrations of phenylalanine.

The results of the amino acid content in the cashmere fibres (Table 3) showed that there were significant differences among the groups in the aspartic acid, methionine, leucine (P<0.05), glutamic acid, glycine, cysteine, tyrosine and arginine contents (P<0.01). The concentrations of aspartic acid, glutamic acid, glycine, cysteine, leucine, tyrosine, arginine (P<0.01) and methionine (P<0.05) were higher in the $ZnSO_4$ group than those in the control group. The cysteine, tyrosine (P<0.01), glutamic acid, glycine and methionine contents (P<0.05) in the HMBi group were higher than those in the control group, but there were no significant differences in the aspartic acid, leucine and arginine contents between the two groups (P>0.05). In addition, the glutamic acid, glycine, leucine and arginine contents in the HMBi group were lower than those in the $ZnSO_4$ group (P<0.05). The cysteine content in the HMBi

Table 2 - Concentrations (mmol/L) of amino acids in plasma

| Items | Treatments | | | P-value |
|---|---|---|---|---|
| | Control | $ZnSO_4$ | HMBi | |
| Aspartic acid | 0.50±0.05 | 0.50±0.03 | 0.51±0.02 | 0.325 |
| Threonine | 0.37±0.04 | 0.38±0.01 | 0.41±0.03 | 0.139 |
| Serine | 0.37±0.05 | 0.39±0.01 | 0.43±0.04 | 0.166 |
| Glutamic acid | 0.73±0.08 | 0.70±0.02 | 0.72±0.03 | 0.361 |
| Glycine | 0.23±0.03 | 0.24±0.02 | 0.26±0.02 | 0.124 |
| Alanine | 0.33±0.04 | 0.34±0.01 | 0.35±0.01 | 0.218 |
| Cysteine | 0.23±0.02 | 0.25±0.01 | 0.23±0.02 | 0.326 |
| Valine | 0.32±0.04 | 0.32±0.02 | 0.34±0.02 | 0.315 |
| Methionine | 0.05±0.01Bb | 0.08±0.01Aa | 0.07±0.01ABa | 0.017 |
| Isoleucine | 0.18±0.02 | 0.19±0.02 | 0.18±0.02 | 0.318 |
| Leucine | 0.73±0.07 | 0.78±0.02 | 0.75±0.06 | 0.226 |
| Tyrosine | 0.05±0.01C | 0.15±0.02A | 0.09±0.01B | <0.001 |
| Phenylalanine | 0.09±0.02Bb | 0.20±0.02Aa | 0.12±0.01Bb | 0.003 |
| Lysine | 0.46±0.07 | 0.44±0.03 | 0.47±0.05 | 0.226 |
| Histidine | 0.18±0.02 | 0.19±0.02 | 0.18±0.01 | 0.351 |
| Arginine | 0.26±0.02 | 0.27±0.01 | 0.26±0.02 | 0.238 |

In the same row, values with different uppercase letters differ significantly (P<0.01); values with different lowercase letters differ significantly (P<0.05).

Table 3 - Contents (mg/g) of amino acids in cashmere fibres

| Items | Treatments | | | P-value |
|---|---|---|---|---|
| | Control | $ZnSO_4$ | HMBi | |
| Aspartic acid | 52.60±2.74Bb | 60.89±2.21Aa | 57.06±2.78ABab | 0.018 |
| Threonine | 50.88±4.00 | 57.38±2.04 | 55.08±2.34 | 0.087 |
| Serine | 86.26±7.58 | 93.52±3.97 | 89.70±1.57 | 0.132 |
| Glutamic acid | 126.13±2.53Bc | 146.47±5.19Aa | 135.32±2.94ABb | 0.005 |
| Glycine | 43.94±2.93Bc | 57.33±1.96Aa | 51.10±2.53ABb | 0.004 |
| Alanine | 30.48±1.30 | 34.83±1.69 | 32.69±1.62 | 0.133 |
| Cysteine | 121.68±2.68C | 132.65±3.11B | 153.44±3.16A | <0.001 |
| Valine | 45.86±3.26 | 46.35±2.36 | 49.65±1.59 | 0.215 |
| Methionine | 5.39±0.32Ab | 6.57±0.28Aa | 6.32±0.66Aa | 0.032 |
| Isoleucine | 25.99±1.80 | 28.07±2.00 | 27.32±1.22 | 0.147 |
| Leucine | 74.10±1.10Bb | 84.07±2.98Aa | 77.64±4.46ABb | 0.022 |
| Tyrosine | 59.84±3.83Bb | 64.14±4.18Aa | 63.85±1.52Aa | 0.004 |
| Phenylalanine | 36.18±3.47 | 37.47±2.56 | 37.20±2.20 | 0.208 |
| Lysine | 29.01±1.60 | 30.36±1.11 | 31.22±2.17 | 0.325 |
| Histidine | 9.21±0.86 | 10.05±0.63 | 10.18±0.86 | 0.216 |
| Arginine | 80.67±1.01Bb | 90.54±3.71Aa | 83.56±1.59ABb | 0.008 |

In the same row, values with different uppercase letters differ significantly (P<0.01); values with different lowercase letters differ significantly (P<0.05).

Table 4 - Sulfur content, growth rate and diameter of cashmere fibres

| Items | Treatments | | | P-value |
|---|---|---|---|---|
| | Control | $ZnSO_4$ | HMBi | |
| Sulfur content (%) | 2.50±0.14Bb | 3.16±0.12Aa | 2.97±0.12Aa | 0.007 |
| Growth rate (mm/d) | 0.27±0.03 Bc | 0.38±0.03Aa | 0.33±0.03Ab | 0.003 |
| Diameter (μm) | 14.02±0.17 | 14.13±0.12 | 13.94±0.08 | 0.103 |

In the same row, values with different uppercase letters differ significantly (P<0.01); values with different lowercase letters differ significantly (P<0.05).

group was higher than that in the $ZnSO_4$ group (P<0.01), whereas no significant differences were observed in the aspartic acid, methionine and tyrosine contents between the two groups (P>0.05). Moreover, there were no significant differences in the concentrations of other amino acids among groups (P>0.05). Thus, supplementation with $ZnSO_4$ or HMBi increases the glutamic acid, glycine, cysteine, methionine and tyrosine contents in cashmere fibres, and the $ZnSO_4$ supplement also increases the aspartic acid, leucine and arginine contents in cashmere fibres.

There were significant differences in the sulfur content of the cashmere fibres (Table 4) among the groups (P<0.01). The sulfur content in the HMBi and $ZnSO_4$ groups was higher than that in the control group (P<0.01), and it was higher in the $ZnSO_4$ group than that in the HMBi group (P<0.01). The results show that $ZnSO_4$ or HMBi supplement increases the sulfur content in the cashmere fibres.

In addition, there were significant differences in the growth rates of the cashmere fibres (Table 4) among the groups (P<0.01). The growth rates in both the HMBi group and the $ZnSO_4$ group were all higher than those in the control group (P<0.01), and they were higher in the $ZnSO_4$ group than those in the HMBi group (P<0.05). However, there were no significant differences in cashmere diameter among the groups (P>0.05). Thus, supplementation with $ZnSO_4$ or HMBi can promote cashmere growth, with no significant effect on cashmere fineness.

## Discussion

Sulfur is an essential element in protein, and its metabolism is closely related to nitrogen metabolism. The plasma concentration of total protein and urea nitrogen can reflect the protein metabolism and the amino acid balance in the animal body (Scott et al., 1982; Chikhou et al., 1993). Limiting amino acid insufficiency or amino acid imbalance can increase the plasma concentration of urea nitrogen in sheep (Wang & Lu, 1999). Wright & Loerch (1988) found that RPMet supplement decreased the plasma concentration of urea nitrogen, and that the plasma methionine concentration increased linearly with the dietary RPMet level. The results of this study indicated that dietary supplementation with $ZnSO_4$ or HMBi increased the concentration of plasma total protein and decreased the concentration of plasma urea nitrogen in cashmere goats. These findings are possibly due to the $ZnSO_4$ or HMBi supplementation promoting the metabolic balance of amino acids and enhancing the metabolism of protein in the goats. Consequently, these results indicate that sulfur supplements can stimulate protein metabolism and increase the rate of dietary protein.

In general, plasma-free amino acid (PFAA) profiles have been used to study the metabolism of proteins, and PFAA serves as an important indicator for studying limiting amino acids. The previous studies found that feeding coated methionine and lysine increased the plasma concentration of free methionine and lysine as a result of the release and the absorption of the amino acids in the intestine (Oke et al., 1986; Robert et al., 1997; Volden et al., 1998). The present study indicated that dietary supplementation with $ZnSO_4$ or HMBi promoted the deposition of some amino acids other than methionine in cashmere goats. Moreover, we found that $ZnSO_4$ supplement increased the plasma concentrations of tyrosine and phenylalanine compared with the HMBi supplement. However, the HMBi supplement had no significant effect on the methionine concentration.

$ZnSO_4$ can provide sulfur to meet the requirements for protein metabolism and methionine production in cashmere goats, and HMBi can be degraded into HMB and then produce methionine via transamination. A previous study detected HMB in circumference blood several minutes after HMBi was infused into the rumen of a cow and the concentration of HMB decreased gradually with an increasing concentration of methionine in blood (Graulet et al., 2005). Consequently, variations in the concentrations of PFAA may reflect the transformation of HMBi in experimental animals and the synergistic effect of HMBi with other amino acids.

Previous studies have reported inconsistent findings on the effects of amino acid supplements on the plasma concentration of amino acids in ruminants. Nimrick et al. (1970) and Bergen (1979) found that the blood concentration of limiting amino acids remained relatively constant until dietary amino acid requirements were met. The increase in the plasma content of amino acids may suggest that they were oversupplied. However, Titgemeyer & Merchen (1990) found that the plasma concentration of amino acids seems to rise markedly when the amount of infused limiting amino acid is slightly lower than dietary needs. Therefore, based on the existing studies, it is difficult to determine whether the HMBi supplement in the present study met the methionine requirements of the cashmere goats of this experiment.

The sulfur content in wool has important effects on the strength, elongation at break and compression resistance of wool (McGuirk, 1983; Qi, 1989). This study indicated that dietary supplement of $ZnSO_4$ or HMBi could increase the amino acid content and the sulfur content in cashmere fibres. The increase in the sulfur content of the cashmere

fibres was probably due to the enhancement of SAA deposition in the cashmere fibres, as noted previously by Xie et al. (2003). Accordingly, it is likely that dietary sulfur supplement has impacts on cashmere quality. Furthermore, this study found that the sulfur content was higher with the $ZnSO_4$ supplement than with the HMBi supplement, demonstrating that $ZnSO_4$ is more important for sulfur retention in cashmere fibres.

Sulfur amino acids are mostly in the form of cystine in wool fibres, but there are only small amounts of cysteine and methionine (Reis & Schinkel, 1963; Reis, 1967; McGuirk, 1983; Reis et al., 1990), and a large amount of cysteine is required for cystine synthesis. Most cystine and cysteine in sheep and goats are absorbed from the gastro-intestinal tract, but the others are transformed by methionine through the trans-sulfuration pathway (Reis, 1989). Thus, the main function of methionine is to provide cysteine for wool protein synthesis (Reis, 1979, 1989). The results of this experiment indicated that dietary supplementation with $ZnSO_4$ or HMBi increases the concentrations of both methionine and cysteine in cashmere fibres. The HMBi supplement was associated with a more significant increase in the cysteine concentration compared with the $ZnSO_4$ supplement. This difference suggests that HMBi supplementation is more beneficial to cysteine deposition in cashmere fibres than the $ZnSO_4$ supplementation. Moreover, we found that the $ZnSO_4$ and HMBi supplements increased the contents of more amino acids in the cashmere fibres than those in plasma. This may be due to the priority in the supply of amino acids to wool growth over other nonfleece-bearing tissues (Langlands et al., 1973). However, Xie et al. (2003) showed that methionine supplementation had no significant effects on the sulfur content or the proportions of SAA, total amino acids and single amino acids in cashmere fibres, but it increased the proportion of total essential amino acids, indicating that methionine supplements can partly promote amino acid deposition in cashmere fibres. At present, few reports regarding the effects of methionine supplements on amino acids other than SAA are available. Hence, further studies should be conducted so as to clarify the effects of methionine supplements on the amino acid composition of cashmere fibres in cashmere goats of different species or ages.

Sulfur is an essential element of keratoprotein which composes cashmere (wool) fibres, and SAA are limiting amino acids in keratoprotein synthesis. Wool growth requires high levels of SAA (Reis, 1989). Bird & Moir (1972) found that methionine infusion through the rumen or abomasums raised the wool yield. The study by Souri et al. (1996) demonstrated that methionine is essential to support the growth and the viability of secondary follicles of the cashmere goats *in vitro*. Moreover, the results of Xie et al. (2003) showed that coated methionine remarkably enhances the growth rate and the length of cashmere fibres in the catagen phase but that it has no significant effects on cashmere fineness. Similarly, this study indicated that dietary supplementation with $ZnSO_4$ or HMBi improved cashmere growth with no effects on its fineness, especially the $ZnSO_4$ supplement. This was probably due to $ZnSO_4$ and HMBi supplements increasing the cysteine content and the raw materials of keratoprotein synthesis, thereby providing optimum conditions for cashmere growth. Neither of the two types of sulfur supplement improved cashmere fineness under the experimental conditions in this study. However, Sahlu & Femendez (1992) found that methionine infusion increased the mean clean mohair yield in Angora goats while increasing the fibre diameter. Similarly, Souri et al. (1998) found that methionine supplementation increased the average diameter of mohair fibres, although it had no significant effects on the cashmere diameter. The differences in the effects of methionine supplementation on fibre fineness in the above studies are most likely due to the animal species, dietary composition and methionine nutritional status.

## Conclusions

Dietary supplementation with $ZnSO_4$ or HMBi can decrease the plasma urea nitrogen concentration and increase concentrations of total protein and methionine in plasma. These sulfur supplements can also increase the content of methionine, cysteine and sulfur in cashmere fibres. Furthermore, the supplements can accelerate cashmere growth with no significant effect on cashmere fineness. The promotion in the cashmere growth of the goats following dietary supplementation with $ZnSO_4$ or HMBi probably results from the improvement in the protein metabolic balance, sulfur retention and sulfur amino acid synthesis. In general, the $ZnSO_4$ supplement can promote greater cashmere growth than the HMBi supplement under the experimental condition.

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