Effects of Dietary 2-Hydroxy-4-(methylthio)butanoic Acid Isopropyl Ester Supplementation Level on Growth Performance, Nutrient Apparent Digestibility, Serum Biochemical Parameters, and Hormone Levels in Goats (Postprint)

Authors: Chen Dong, Li Siyuan, Meiqing Chen, Zhang Peihua, Li Xinhua, Mi Hui, Shen Weijun, Zhang Qian, Li Siwen

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary isopropyl 2-hydroxy-4-(methylthio)butanoate (HMBi) supplementation levels on growth performance, apparent nutrient digestibility, serum biochemical indices, and hormone levels in goats, in order to determine the optimal dietary HMBi supplementation level for goats. Thirty-six goats at approximately 2.5 months of age with an average body weight of (13.33±1.71) kg were selected and randomly divided into 4 groups, with 3 replicates per group and 3 goats per replicate (1 castrated male and 2 females). Goats in each group were fed experimental diets with HMBi supplementation levels of 0 (control group), 0.05%, 0.10%, and 0.20% (dry matter basis). The experiment consisted of a 10-day preliminary period and a 40-day formal experimental period. The results showed: 1) The 0.10% group exhibited the highest average daily gain (158.89 g/d), lowest feed-to-gain ratio (4.82), and optimal economic benefit, with both average daily gain and gross profit being significantly higher than those of the control group ($P<0.05$). The average daily gains of the remaining groups were as follows: 0.20% group, 141.11 g/d; 0.05% group, 140.56 g/d; control group, 119.44 g/d. 2) No significant differences were observed among groups in the apparent digestibility of dry matter, neutral detergent fiber, acid detergent fiber, and digestible energy ($P>0.05$). The apparent digestibility of crude protein in the 0.10% group was significantly higher than that in the control group ($P<0.05$). 3) On day 1 of the experiment, no significant differences were detected among groups in serum urea nitrogen (UN), glucose (GLU), triglycerides (TG), total cholesterol (TC), non-esterified fatty acids (NEFA), total amino acids (TAA) contents, or growth hormone (GH) and insulin-like growth factor-I (IGF-I) levels ($P>0.05$). On day

31 of the experiment, serum TG content in the 0.20% group was significantly higher than that in the other three groups (P<0.05); serum TC content in the 0.20% group was significantly lower than that in the control group (P<0.05); serum NEFA content in the 0.20% group was extremely significantly higher than that in the control group (P<0.01) and significantly higher than that in the 0.05% group (P<0.05); serum TAA contents in the 0.05%, 0.10%, and 0.20% groups were significantly higher than that in the control group (P<0.05); serum GH levels in the 0.05% and 0.10% groups were significantly higher than that in the control group (P<0.05), while serum GH level in the 0.20% group showed an increasing trend compared with the control group (P=0.084); no significant differences were observed in serum IGF-I levels among groups (P>0.05). These results indicate that dietary HMBi supplementation is beneficial for improving protein digestion and utilization in goats, promoting goat growth, and that a supplementation level of 0.10% (dry matter basis) yields the optimal economic benefit.

Full Text

Effects of 2-Hydroxy-4-(Methylthio) Butanoic Acid Isopropyl Ester Supplemental Level on Growth Performance, Nutrient Apparent Digestibility, Serum Biochemical Indices and Hormone Levels of Goats

CHEN Dong[1], LI Siyuan[1], *CHEN Meiqing[1], ZHANG Peihua[1]*, LI Xinhua[1], MI Hui[1], SHEN Weijun[1], ZHANG Qian[2], LI Siwen[3]

[1]College of Animal Science and Technology, Hunan Agricultural University, Changsha 410123, China
[2]Adisseo Life Science (Shanghai) Co., Ltd., Shanghai 200127, China
[3]Chaling Huiwang Agriculture and Animal Husbandry Development Co., Ltd., Zhuzhou 412000, China

Corresponding authors: LI Siyuan, senior experimentalist, E-mail: lsy1961@126.com; ZHANG Peihua, associate professor, E-mail: 540517641@qq.com

---

Abstract

This experiment was conducted to investigate the effects of dietary 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester (HMBi) supplementation level on growth performance, nutrient apparent digestibility, serum biochemical indices, and hormone levels in goats, aiming to determine the optimal HMBi supplemental level. Thirty-six goats approximately 2.5 months old with an average body weight of (13.33±1.71) kg were randomly assigned to four groups, with three replicates per group and three goats per replicate (one castrated male and two females). Each group was fed experimental diets containing 0 (control), 0.05%, 0.10%, or 0.20% HMBi on a dry matter basis. The pre-trial period lasted 10 days, followed by a 40-day formal experiment. The results showed: (1) The 0.10% group achieved the highest average daily gain (ADG) of 158.89 g/d, the lowest feed-to-gain ratio of 4.82, and the best economic benefit. Both ADG and gross returns in the 0.10% group were significantly higher than those in the control group ($P<0.05$). The ADG values for the other groups were: 0.20% group, 141.11 g/d; 0.05% group, 140.56 g/d; and control group, 119.44 g/d. (2) No significant differences were observed among groups in apparent digestibility of dry matter, neutral detergent fiber, acid detergent fiber, or digestible energy ($P>0.05$). The crude protein apparent digestibility in the 0.10% group was significantly higher than that in the control group ($P<0.05$). (3) On day 1 of the experiment, no significant differences were detected among groups in serum urea nitrogen (UN), glucose (GLU), triglycerides (TG), total cholesterol (TC), non-esterified fatty acid (NEFA), total amino acid (TAA) content, or growth hormone (GH) and insulin-like growth factor-I (IGF-I) levels ($P>0.05$). On day 31, the 0.20% group exhibited significantly higher serum TG content than the other three groups ($P<0.05$); serum TC content in the 0.20% group was significantly lower than in the control group ($P<0.05$); serum NEFA content in the 0.20% group was extremely significantly higher than in the control group ($P<0.01$) and significantly higher than in the 0.05% group ($P<0.05$); serum TAA content in the 0.05%, 0.10%, and 0.20% groups was significantly higher than in the control group ($P<0.05$); serum GH levels in the 0.05% and 0.10% groups were significantly higher than in the control group ($P<0.05$), while the 0.20% group showed an increasing trend compared with the control group ($P=0.084$); no significant differences were observed in serum IGF-I levels among groups ($P>0.05$). These results indicate that dietary HMBi supplementation improves protein digestion and utilization in goats and promotes growth, with a supplemental level of 0.10% (dry matter basis) providing the best economic benefits.

Keywords: goat; HMBi; supplemental level; growth performance; serum biochemical indices; hormone

Introduction

Dietary protein or amino acids in ruminants are partially degraded in the rumen. The rumen-undegraded amino acids and microbial protein are subsequently absorbed and converted in the small intestine, where absorbed amino acids are crucial for maintaining vital activities such as growth, fattening, and reproduction. Previous studies have confirmed that methionine (Met), an important limiting amino acid for ruminants, plays a significant role in maximizing animal production potential and alleviating the shortage of protein feed resources. Methionine participates extensively in animal growth and metabolic processes, yet it is one of the most deficient amino acids in feed ingredients such as soybean meal. Direct dietary supplementation of methionine is partially or completely degraded by rumen microorganisms, resulting in limited amounts reaching the small intestine for absorption. Therefore, increasing the content of rumen-protected methionine and its absorption rate in the small intestine is particularly important in ruminant nutrition. Research has shown that adding rumen-protected methionine to feed can increase the amount of methionine reaching the small intestine, improve absorption rates, overcome amino acid imbalances caused by essential amino acid shortages, and enhance ruminant production performance. Current rumen protection methods for amino acids mainly include physical coating and chemical protection, with physically coated rumen-protected methionine being predominantly used in sheep studies.

2-Hydroxy-4-(methylthio)butyric acid (HMB) is a methionine hydroxy analog that is easily degraded in the rumen of ruminants, with degradation rates ranging from 50% to 99%. Consequently, HMB is not an ideal methionine additive for ruminant production. 2-Hydroxy-4-(methylthio)butanoic acid isopropyl ester (HMBi) is produced through esterification of HMB with isopropanol. In ruminants, HMBi is rapidly absorbed through the rumen wall, then decomposed into HMB, which is subsequently converted to methionine via transamination. This process compensates for the disadvantage of HMB being easily degraded by rumen microorganisms, making HMBi a methionine additive with high biological value. Current research and application of HMBi in ruminants have primarily focused on dairy cows, with general consensus that dietary HMBi supplementation can increase milk yield, milk protein percentage, and milk fat percentage. However, few studies have reported on HMBi supplementation in sheep. Therefore, this experiment investigated the effects of dietary HMBi supplementation level on growth performance, nutrient apparent digestibility, and serum biochemical indices and hormone levels in goats to determine the optimal supplemental level, providing a reference for applying HMBi as a feed additive in growing goats.

1.1 Experimental Animals and Diets

Thirty-six healthy Liuyang black goats approximately 2.5 months old, with an average body weight of (13.33±1.71) kg, were selected from the Liuyang Chunfeng Breeding Cooperative in Changsha. Using a single-factor experimental design, the goats were randomly divided into four groups with three replicates per group and three goats per replicate (one castrated male and two females). The experimental diets were formulated to meet the nutritional requirements of fattening goats with 15 kg body weight and average daily gain of 110 g/d, according to NRC (2007) and China's "Feeding Standard of Meat Sheep" (NY/T 816-2004). The composition and nutrient levels of the experimental diets are shown in Table 1 . Each group was fed experimental diets containing 0 (control), 0.05%, 0.10%, or 0.20% HMBi on a dry matter basis. All experimental diets were prepared as total mixed pellets (pellet diameter 8 mm, length 10 mm). The costs of these experimental diets were 1,901.1, 1,938.6, 1,976.1, and 2,051.1 yuan/ton, respectively, with a live goat price of 25 yuan/kg.

1.2 Animal Management

The experiment was conducted at the Liuyang Chunfeng Breeding Cooperative in Changsha. After a 10-day pre-feeding period, the formal 30-day experiment began. All experimental goats were ear-tagged upon purchase, vaccinated with a triple vaccine before pre-feeding, and dewormed with ivermectin solution (2.5 mL/head). Goats were housed in groups of three per pen (12 m^2 per pen) and fed at 08:00 and 17:00 daily. Feed amounts were adjusted daily based on residual feed in the trough from the previous day, ensuring approximately 10% leftover feed daily, with free access to water. After the feeding trial, six goats with similar body condition from each group (three castrated males and three females) were selected and placed in metabolic cages with free water access for a 5-day digestion trial. Daily feed intake was accurately recorded, and fecal output was recorded and sampled using the total collection method. The diet composition and feeding method during the digestion trial were identical to those in the feeding trial.

1.3 Measurements

1.3.1 Growth Performance Measurement Body weight was measured before morning feeding at the beginning and end of the experiment as initial and final weight, respectively, to calculate average daily gain (ADG). Residual feed was cleaned and weighed daily before feeding to calculate average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was calculated based on ADFI and ADG. Economic benefit was measured as gross returns, calculated as daily weight gain income minus daily feed cost.

ADG (g/d) = [(final weight - initial weight) / experimental days] × 1000
ADFI (g/d) = (total feed intake during formal period / experimental days) × 1000
Feed-to-gain ratio = ADFI / ADG
Gross returns [yuan/(head · d)] = weight gain income - feed cost

1.3.2 Nutrient Apparent Digestibility Measurement During the digestion trial, feces were collected, weighed, and recorded at 08:00 and 17:00 daily. Diet samples were randomly collected before daily feeding, and the 5-day samples were mixed for subsequent analysis. Diet and fecal samples were analyzed for gross energy (GE), dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), and phosphorus (P) content according to "Feed Analysis and Feed Quality Detection Technology". The apparent digestibility of each nutrient was calculated as follows:

Apparent digestibility of a nutrient (%) = [(nutrient content in consumed feed - nutrient content in feces) / nutrient content in consumed feed] × 100

1.3.3 Serum Biochemical Indices and Hormone Levels Measurement

On days 1 and 31 of the experiment, three female goats were randomly selected from each group, and 10 mL of blood was collected from the jugular vein before morning feeding. After standing for 15 minutes, serum was separated by centrifugation at 3,000 r/min for 10 minutes and stored at -20°C for subsequent analysis. Serum biochemical indices included urea nitrogen (UN), glucose (GLU), triglycerides (TG), total cholesterol (TC), non-esterified fatty acid (NEFA), and total amino acid (TAA) content. Serum hormone indices included growth hormone (GH) and insulin-like growth factor-I (IGF-I). All indices were measured according to kit instructions provided by Beijing Andihuatai Biotechnology Co., Ltd.

1.4 Statistical Analysis

Experimental data were initially processed using Excel 2007 and expressed as mean ± standard deviation. Statistical analysis was performed using SAS 9.0 software with one-way ANOVA procedure, and Duncan' s multiple comparison test was used. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

2.1 Effects of Dietary HMBi Supplemental Level on Growth Performance and Economic Benefit of Goats

As shown in Table 2 , no significant differences were observed among the four groups in initial weight, final weight, or average daily feed intake ($P>0.05$). The ADG of goats in the 0.10% group was significantly higher than that in the control group ($P<0.05$), with ADG values of 158.89 g/d in the 0.10% group and 119.44 g/d in the control group. The feed-to-gain ratio in the 0.10% group was significantly lower than that in the control group ($P<0.05$), with values of 4.82 and 6.61, respectively. No significant differences in ADG or feed-to-gain ratio were observed between the 0.05% and 0.20% groups and the control group ($P>0.05$).

As shown in Table 3 , after HMBi supplementation, no significant differences were found in feed cost among groups ($P>0.05$). Following the feeding trial, the 0.10% group achieved the highest weight gain income and gross returns, both significantly higher than those in the control group ($P<0.05$). The weight gain income in the 0.10% group was 3.97 yuan/(head·d), which was 0.98 yuan/(head·d) higher than the control group. The gross returns in the 0.10% group were 2.35 yuan/(head·d), 0.79 yuan/(head·d) higher than the control group. The 0.05% and 0.20% groups ranked second in weight gain income and gross returns, while the control group showed the lowest values.

2.2 Effects of Dietary HMBi Supplemental Level on Nutrient Apparent Digestibility of Goats

As shown in Table 4 , no significant differences were observed among groups in apparent digestibility of dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), or digestible energy (DE) (P>0.05). The 0.10% group exhibited the highest crude protein (CP) apparent digestibility at 75.86%, which was significantly higher than that of the control group (P<0.05). The 0.05% and 0.20% groups showed intermediate CP apparent digestibility values of 72.76% and 71.59%, respectively, while the control group had the lowest value at 69.68%.

2.3 Effects of Dietary HMBi Supplemental Level on Serum Biochemical Indices of Goats

As shown in Table 5 , on day 1 of the experiment, no significant differences were observed among groups in serum UN, GLU, TG, TC, NEFA, or TAA content (P>0.05). On day 31, serum TAA content in the 0.05%, 0.10%, and 0.20% groups was significantly higher than in the control group (P<0.05), with no significant differences among the three supplemented groups (P>0.05), indicating that dietary HMBi supplementation significantly increased serum TAA content in goats. On day 31, serum TG content in the 0.20% group was 0.93 mmol/L, extremely significantly higher than in the 0.10% group (P<0.01) and significantly higher than the other three groups (P<0.05). The 0.10% group had the lowest serum TG content at 0.77 mmol/L. Serum TC content in the 0.20% group was 2.47 mmol/L on day 31, significantly lower than in the control group (P<0.05), with no significant differences among other groups (P>0.05). Serum NEFA content in the 0.20% group was 0.39 mmol/L on day 31, extremely significantly higher than in the control group (P<0.01) and significantly higher than in the 0.05% group (P<0.05), with no significant differences among other groups (P>0.05).

2.4 Effects of Dietary HMBi Supplemental Level on Serum Hormone Levels of Goats

As shown in Table 6 , on day 1 of the experiment, no significant differences were observed among groups in serum GH or IGF-I levels (P>0.05). On day 31, serum GH levels in the 0.05% and 0.10% groups were significantly higher than in the control group (P<0.05), while the 0.20% group showed an increasing trend compared with the control group (P=0.084), indicating that dietary HMBi supplementation increased serum GH levels in goats. No significant differences in serum IGF-I levels were observed among groups on either day 1 or day 31 (P>0.05).

Discussion

3.1 Effects of Dietary HMBi Supplemental Level on Growth Performance of Goats

Previous studies have shown that supplementation with different forms of methionine can improve weight gain and economic benefits in animals. For example, Tang et al. found that adding HMBi to calf starter increased total weight gain and daily weight gain by 14.82% and 14.93%, respectively, compared with the control group, though the differences were not significant. Wang et al. reported that supplementation with rumen-protected methionine (coated with palm oil, containing approximately 40% methionine) in the diet of housed fattening Tan sheep lambs significantly improved total weight gain and promoted production performance. Yan et al. observed that adding rumen-protected methionine (coated with animal oil, containing approximately 70% methionine) to the diet of 9-month-old Small-tailed Han sheep significantly increased ADG, with the 0.485% supplemental group showing extremely significantly higher ADG than the control group. Han et al. found that HMBi supplementation at 15 and 25 g/(d • head) in the diet of growing Holstein steers extremely significantly increased final weight, total weight gain, and ADG. Xi et al. reported that HMBi supplementation in dairy cows [5 g/(d • head) during prepartum period and 10 g/(d • head) during peak lactation] significantly improved milk production performance and milk quality, thereby enhancing economic benefits. The present study demonstrated that dietary HMBi supplementation promoted goat growth, with the 0.10% supplemental level showing significantly higher ADG and feed-to-gain ratio compared with the control group. However, when the supplemental level increased to 0.20%, weight gain speed decreased compared with the 0.10% level. This is primarily because most dietary protein is degraded by rumen microorganisms, with only a small portion absorbed and utilized by the small intestine. Supplementing rumen-protected amino acids in the diet is an effective way to improve dietary protein levels for ruminants. The present results are consistent with previous findings. The reduced growth rate at HMBi levels exceeding the optimal amount may be explained by two factors: first, when methionine supply exceeds the animal' s requirement per unit metabolic body weight, the animal must expend its own energy to catabolize excess amino acids; second, excess amino acids can cause protein metabolism disorders, thereby affecting animal growth and development. Ouyang et al. reported that high doses of methionine significantly increased glycine methyltransferase activity in rats, leading to excessive consumption of glycine and ultimately causing amino acid imbalance.

3.2 Effects of Dietary HMBi Supplemental Level on Nutrient Apparent Digestibility of Goats

Eltahawy et al. found that supplementation with 3.30 g rumen-protected methionine per kilogram of concentrate in lambs significantly improved weight gain, with significantly higher apparent digestibility of DM, organic matter (OM), CP, EE, and nitrogen-free extract (NFE) compared with the control group. Wang et al. reported that rumen-protected methionine supplementation (containing >84% methionine) in meat sheep diets did not significantly change apparent digestibility of DM, CP, EE, NDF, or ADF. Baghbanzadeh-Nobari et al. observed that HMBi supplementation in ewe diets at 1.8 g/kg DM significantly improved apparent digestibility of OM, CP, and NDF, with a trend toward improved DM and ADF apparent digestibility. The present study showed that the 0.10% group had significantly higher CP apparent digestibility than the control group, indicating that 0.10% HMBi supplementation increased CP apparent digestibility and consequently produced the best weight gain results. When dietary HMBi supplemental level reached 0.20%, no significant differences were observed in nutrient apparent digestibility compared with the control group, suggesting that HMBi supplementation up to 0.20% did not adversely affect nutrient apparent digestibility in goats.

3.3 Effects of Dietary HMBi Supplemental Level on Serum Biochemical Indices of Goats

Serum UN is a product of protein metabolism and can reflect protein metabolism status and dietary amino acid balance to some extent. Protein utilization efficiency and amino acid balance determine serum UN content—when protein utilization is high or amino acids are balanced, serum UN content decreases, and vice versa. Additionally, serum UN content is closely correlated with nitrogen deposition. The liver is the primary organ for amino acid metabolism, and serum TAA content can reflect protein and amino acid metabolism status as well as liver health. McCollum et al. found that liquid HMB supplementation in sheep diets enhanced nitrogen absorption capacity. Oke et al. reported that rumen-protected methionine supplementation in sheep diets increased nitrogen deposition with increasing supplemental levels. Baghbanzadeh-Nobari et al. observed that HMBi supplementation in ewe diets at 1.8 g/kg DM significantly decreased serum UN content. Feng et al. found that HMBi supplementation at 1.27% in 6-month-old Liaoning cashmere goats significantly reduced plasma UN content. Xia et al. reported that HMBi supplementation in dry period dairy cows did not significantly affect serum TAA content. The present study showed that HMBi supplementation did not significantly reduce serum UN content in goats, possibly due to differences in supplemental levels, basal diets, and animal conditions. However, serum UN content tended to decrease with increasing HMBi supplemental levels, suggesting a potential effect. All three supplemented groups showed significantly increased serum TAA content compared with the control group. The changes in serum UN and TAA content further support the growth-promoting effect of HMBi supplementation in goats.

Serum GLU, TG, TC, and NEFA are important indicators related to glucose and lipid metabolism, reflecting energy homeostasis. Serum GLU content is positively correlated with growth rate to some extent. Serum TC content reflects lipid metabolism status, while serum TG, as a product of fat metabolism, is a direct indicator of fat digestion and absorption—higher content indicates lower fat utilization efficiency. NEFA is a product of fat hydrolysis; higher content indicates stronger fat mobilization. During fat breakdown, large amounts of NEFA are produced and enter the blood and liver, where they can be used to synthesize TG. Baghbanzadeh-Nobari et al. found that HMBi supplementation in ewe diets at 1.8 g/kg DM significantly increased serum GLU content and decreased serum TG content. Tang et al. and Wang et al. both reported that HMBi-supplemented groups had extremely significantly lower serum TG content than the control group in the later experimental period, indicating that HMBi benefits fat deposition in goats. Xi et al. found that HMBi supplementation in dairy cows did not significantly change serum NEFA, TC, or TG content, though serum NEFA and TC tended to decrease. The present study found that HMBi supplementation did not significantly affect serum GLU content in goats, consistent with Xi et al.' s findings in dry period dairy cows. On day 1, no significant differences were observed among groups in serum NEFA, TG, or TC content. On day 31, the 0.20% group showed significantly higher serum TG content than the other three groups, significantly lower serum TC content than the control group, and extremely significantly higher serum NEFA content than the control group and significantly higher than the 0.05% group. These results indicate that HMBi supplementation significantly or extremely significantly increased serum NEFA content, significantly increased serum TG content, and significantly decreased serum TC content in goats, which is consistent with the growth performance results.

3.4 Effects of Dietary HMBi Supplemental Level on Serum Hormone Levels of Goats

The growth-promoting effect of GH is mediated by IGF-I, which can amplify GH' s growth-promoting effects. Rausch et al. demonstrated that exogenous GH injection in beef cattle increased growth rate, improved feed conversion efficiency, and elevated serum IGF-I levels. Geng et al. found that rumen-protected methionine supplementation in Liaoning cashmere goats significantly increased blood GH levels, with the 10 g/d supplemental level having the greatest effect on blood hormone levels. Han et al. reported that HMBi supplementation at 15 g/(d · head) in growing Holstein steers extremely significantly increased serum GH levels. The present study found no significant differences in serum GH or IGF-I levels among groups on day 1. On day 31, serum GH levels in the 0.05% and 0.10% groups were significantly higher than in the control group, consistent with Han et al.' s findings and indicating that HMBi supplementation promotes goat growth. However, HMBi supplementation did not significantly affect serum IGF-I levels in goats, which differs from Rausch et al.' s results. The mechanism by which HMBi affects serum hormone levels in goats remains unclear and warrants further investigation.

Conclusion

1. Compared with the control group, dietary supplementation with 0.10% HMBi significantly improved ADG and provided better economic benefits in goats.
2. Compared with the control group, dietary supplementation with 0.10% HMBi significantly increased CP apparent digestibility, serum TAA content, and GH levels in goats.

References

[1] DONG Xianwen, ZUO Fuyuan. Research and application of rumen-protected methionine[J]. *Feed China*, 2012(8): 30-32.

[2] YANG Zhilin, LI Beibei, FENG Jianfang. Application of rumen-protected amino acids in ruminant production[J]. *Feed Review*, 2016(8): 18-20.

[3] HU Chengjun, JIANG Qingyan, KONG Xiangfeng. Research progress on methionine metabolism and physiological functions in livestock and poultry[J]. *Feed Industry*, 2016, 37(15): 23-27.

[4] ZHU Zhongsheng, LI Lümu. Research progress on methionine[J]. *Feed Review*, 2015(7): 11-17.

[5] LU Yufei, ZHANG Xueyuan, MA Tingting, et al. Research progress on rumen-protected methionine in ruminants[J]. *Feed Industry*, 2014, 35(17): 13-18.

[6] CHU Yongkang, LIN Yingting, CHEN Qiaoqiao. Application of rumen-protected amino acids in ruminant feed[J]. *China Feed*, 2012(6): 36-39.

[7] JONES B A, MOHAMED O E, PRANGE R W, et al. Degradation of methionine hydroxy analog in the rumen of lactating cows[J]. *Journal of Dairy Science*, 1988, 71(2): 525-529.

[8] KOENIG K M, RODE L M, KNIGHT C D, et al. Ruminal escape, gastrointestinal absorption, and response of serum methionine to supplementation of liquid methionine hydroxy analog in dairy cows[J]. *Journal of Dairy Science*, 1999, 82(2): 355-361.

[9] GRAULET B, RICHARD C, ROBERT J C. The isopropyl ester of methionine hydroxy-analogue is absorbed through the rumen wall in the cow[J]. *Journal of Animal & Feed Sciences*, 2004, 13(1S): 269-272.

[10] LIN Yi, WANG Zhisheng, ZHOU Anguo. Application of 2-hydroxy-4-(methylthio)butanoic acid isopropyl ester (HMBi) and 2-hydroxy-4-(methylthio)butanoic acid (HMB) in ruminants[J]. *China Dairy Cattle*, 2008(11): 11-15.

[11] YANG Baokui, REHE Mutayi, XI Yumeng, et al. Effects of methionine hydroxy analog isopropyl ester on production performance and serum biochemical indices of dairy cows[J]. *Journal of Fujian Agriculture and Forestry University (Natural Science Edition)*, 2017, 46(1): 89–94.

[12] XIE Xiaofeng, ZHOU Yuming, WANG Mingliang. Effects of dietary rumen-protected methionine on production performance of dairy cows[J]. *Animal Husbandry & Veterinary Medicine*, 2014, 46(4): 50–51.

[13] ZHANG Liying. *Feed Analysis and Feed Quality Detection Technology*[M]. 3rd ed. Beijing: China Agricultural University Press, 2007.

[14] TANG Bo, WANG Qun, XI Yumeng, et al. Effects of methionine hydroxy isopropyl ester on growth, serum biochemical indices and hormone levels in calves[J]. *Jiangsu Journal of Agricultural Sciences*, 2014(3): 567–573.

[15] WANG Meng, ZHOU Yuxiang, ZHANG Yanmei, et al. Effects of rumen-protected methionine on production performance of housed Tan sheep[J]. *Journal of Domestic Animal Ecology*, 2017, 38(1): 36–38.

[16] YAN Lei, YANG Weiren, YANG Zaibin, et al. Effects of different levels of rumen-protected methionine on nitrogen metabolism and production performance of Small-tailed Han sheep[J]. *Journal of Domestic Animal Ecology*, 2005, 26(6): 27–30.

[17] HAN Z Y, YANG B K, YANG Z, et al. Effects of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester on growth and blood components in growing Holstein steers[J]. *Animal Science Journal*, 2016, 88(2): 286–293.

[18] XI Weibin, XIA Ke, ZHANG Yonggen, et al. Effects of balanced lysine and methionine on production performance and blood biochemical indices of dairy cows[C]// ZHANG Yuan. *Healthy Development and Scientific Innovation of Chinese Cattle Industry—Proceedings of the 7th Academic Symposium of Cattle Branch of Chinese Association of Animal Science and Veterinary Medicine, 2009*. Nanjing: Nanjing Agricultural University Dairy Science Research Institute, 2009: 101.

[19] OUYANG Peizhen, TANG Yangbo. Effects of excess methionine on 3H-leucine incorporation in rat liver and brain tissues[J]. *Acta Nutrimenta Sinica*, 1993(3): 304–308.

[20] ELTAHAWY A S, ISMAEIL A M. Methionine-supplemented diet increases the general performance value of Rahmani lambs[J]. *Iranian Journal of Applied Animal Science*, 2013, 3: 513–520.

[21] WANG Huiyuan, ZHANG Yingjie, LIU Yueqin, et al. Study on production performance and nutrient digestibility of meat sheep supplemented with rumen-protected methionine[J]. *Feed Industry*, 2014, 35(3): 51–54.

[22] BAGHBANZADEH-NOBARI B, TAGHIZADEH A, KHORVASH M, et al. Digestibility, ruminal fermentation, blood metabolites and antioxidant sta-

tus supplemented with DL-methionine or hydroxy-4-(methylthio) butanoic acid isopropyl ester[J]. *Journal of Animal Physiology and Animal Nutrition*, 2017, 101(5): 266–277.

[23] STANLEY C C, WILLIAMS C C, JENNY B F, et al. Effects of feeding milk replacer once versus twice daily on glucose metabolism in holstein and jersey calves[J]. *Journal of Dairy Science*, 2002, 85(9): 2335–2343.

[24] YAN Changguo, JIN Zelin, JIN Taihua, et al. Experimental study of exogenous growth hormone in Yanbian cattle ( )—Effects on blood physiological and biochemical indices[J]. *Chinese Journal of Animal Science*, 2005, 41(3): 6–9.

[25] MCCOLLUM M Q, VÁZQUEZ-AÑÓN M, DIBNER J J, et al. Absorption of 2-hydroxy-4-(methylthio) butanoic acid by isolated sheep ruminal and omasal epithelia[J]. *Journal of Animal Science*, 2000, 78(4): 1078–1083.

[26] OKE B O, LOERCH S C, DEETZ L E. Effects of rumen-protected methionine and lysine on ruminant performance and nutrient metabolism[J]. *Journal of Animal Science*, 1986, 62(4): 1101–1112.

[27] FENG Y L, SUN Y, DENG H W, et al. Promotion of cashmere growth by sulfur supplements in cashmere goats[J]. *Revista Brasileira De Zootecnia*, 2013, 42(11): 792–797.

[28] XIA Ke, ZHANG Yonggen, WANG Yan, et al. Effects of dietary HMBi on blood indices and health status of dairy cows[J]. *China Feed*, 2010(12): 9–12.

[29] KNOWLES T A, SOUTHERN L L, BIDNER T D, et al. Effect of dietary fiber or fat in low-crude protein, crystalline amino acid-supplemented diets for finishing pigs[J]. *Journal of Animal Science*, 1998, 76(11): 2818–2832.

[30] WANG Qun, YIN Junli, TANG Bo, et al. Effects of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester supplementation in diets with different protein levels on production performance and blood biochemical indices of dairy cows[J]. *Journal of Nanjing Agricultural University*, 2014, 37(3): 126–132.

[31] SUN Wei, MA Wenjian, SONG Enliang, et al. Effects of feeding different levels of heat-treated soybean on insulin-like growth factor-I, growth hormone, insulin and leptin levels in body tissues and serum of beef cattle[J]. *Grass-Feeding Livestock*, 2011(2): 35–39.

[32] RAUSCH M I, TRIPP M W, GOVONI K E, et al. The influence of level of feeding on growth and serum insulin-like growth factor I and insulin-like growth factor-binding proteins in growing cattle supplemented with somatotropin[J]. *Journal of Animal Science*, 2002, 80(1): 94–100.

[33] GENG Zhongcheng, LIU Lili, ZHANG Hu, et al. Effects of rumen-protected methionine on blood hormones in cashmere goats[J]. *Journal of Heilongjiang Bayi Agricultural University*, 2011, 23(2): 20–23.

Note: Figure translations are in progress. See original paper for figures.