Effects of betaine on growth performance, serum biochemical indices, slaughter performance and intramuscular fat deposition in finishing Small-tailed Han sheep

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Abstract

This study aimed to investigate the effects of supplementing diets with different concentrations of betaine on the growth performance, blood biochemical indices, slaughter performance, and intramuscular fat content of fattening Small-tailed Han sheep. Forty weaned male lambs, with an average body weight of 15.58 ± 2.86 kg, were randomly assigned to one of four groups: Control, a low-dose betaine group (BET-L; 1 g/day per lamb), a medium-dose group (BET-M; 3 g/day per lamb), and a high-dose group (BET-H; 5 g/day per lamb). They were raised in individual pens. The experimental period consisted of a 15-day preliminary adaptation phase followed by a 153-day feeding trial. No significant differences were found in pre-slaughter body weight, carcass weight, or dressing percentage ($P > 0.05$). Crude protein and water content in the longissimus dorsi muscle were comparable across all groups ($P > 0.05$). However, the BET-H group demonstrated a significantly higher fat content than the other groups ($P < 0.05$). Gene expression analysis of the longissimus dorsi muscle in the two groups with a significantly different fat content revealed no significant differences in mRNA expression of heart-type fatty acid-binding protein (*H-FABP*), fatty acid synthase (*FAS*), peroxisome proliferator-activated receptor α (*PPARα*) and hormone-sensitive triglyceride lipase (*HSL*), but the BET-H group showed a significant increase ($P < 0.05$) in lipoprotein lipase (*LPL*) expression compared to the control group. Dietary supplementation with betaine at a dose of 5 g/day per lamb enhanced fat accumulation in the longissimus dorsi muscle of fattening lambs and improved meat quality by the upregulation of the *LPL* gene encoding a key enzyme in fat synthesis.

Ovine, meat performance, m. longissimus dorsi, lipoprotein lipase, gene expression

In animal farming, meat quality is a key economic trait, prompting significant research efforts to enhance both meat yield and quality. Feed plays a crucial role in improving growth efficiency and slaughter outcomes, directly influencing the economic viability and market competitiveness of sheep farming. Consequently, identifying feed additives that optimize these indices has become a prominent research focus.

Betaine, scientifically known as trimethylglycine, functions as an efficient methyl donor and plays a key role in protein and lipid metabolism. Since animals have limited capacity to synthesize methyl groups and feed sources are typically low in betaine, supplementation is often required (Yang et al. 2020). Most studies have demonstrated that betaine enhances the growth and slaughter performance of livestock and poultry, regulates fat distribution, reduces overall body fat, and increases intramuscular fat content (Martins et al. 2010; Figueroa-Soto and Valenzuela-Soto 2018; Chen et al. 2020). For instance, supplementation of piglet feed with betaine improves growth performance, promotes protein deposition, and stimulates fat catabolism (Fu et al. 2022). Lothong et al. (2013) indicated that betaine supplementation reduced back-fat thickness in piglets, while Fu et al. (2023) reported improvements in both daily feed intake and growth efficiency in pigs following betaine supplementation. In a study involving Holstein bulls, betaine was found to improve growth performance, nutrient digestion, and rumen fermentation (Lei et al. 2024).

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Despite these findings, there has been limited research examining the effects of betaine on growth performance, slaughter quality, blood biochemical properties, and fat deposition in the longissimus dorsi muscle in Small-tailed Han weaned male lambs during the fattening period.

Therefore, this pilot study was designed to investigate the impact of different doses of betaine added to the feed of Small-tailed Han fattening lambs on their growth performance, slaughter outcomes, blood biochemical indicators, and muscle fat accumulation. The aim was to provide theoretical support for the rational application of betaine as a feed additive and the optimization of meat quality in fattening lambs.

Materials and Methods

Ethical approval

This study was approved by the Ethics Committee of Shanxi Agricultural University and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals

Forty healthy Small-tailed Han weaned male lambs, approximately 3 months of age and with an average body weight of 15.58 ± 2.86 kg, were selected from a breeding facility located in Huairen City, Shanxi Province.

Rations

The basal diet was designed to provide the essential nutrients required for the growth and development of fattening lambs expected to reach a body weight of 20–45 kg. The formulation was based on established nutritional guidelines and the availability of local forage materials, as detailed in Table 1 (Mao et al. 2021). Betaine was supplemented at doses of 1, 3, and 5 g/day per lamb in the respective test groups, while no betaine was added to the control group (CON).

Table 1. Feed composition and nutritional level of the fattening lambs' basal diet.

Ingredient	Content (%)	Nutritional level	Content (%)
Concentrated feed	16.53	Crude protein	13.30
Corn	49.95	Digestible energy	14.04
Whole corn silage	22.41	Neutral detergent fibre	22.09
Alfalfa	8.98	Calcium	0.70
Peanut vine	2.13	Phosphorus	0.24
Total	100.00		

The concentrated feed consisted of 57% soybean meal, 12% sesame cake, 12% premix, 9% soybean oil, 2% calcium hydrogen phosphate ($CaHPO_4$), 2% sodium chloride (NaCl), and 6% sodium bicarbonate ($NaHCO_3$). The premix contained the following per kilogram: 200,000 IU of vitamin A, 70,000 IU of vitamin D_3, 750 mg of vitamin E, 1,000 mg of iron, 1,000 mg of manganese, 1,000 mg of zinc, 380 mg of copper, 100 g of calcium, and 100 g of NaCl. The digestible energy was calculated based on the feed composition formula, while other nutritional indicators were determined through direct measurement.

Main reagents and instruments

Rumen-protected betaine was procured from Beijing Challenge Bio-Technology Co., Ltd. (Beijing, China). The RNA extraction kit (Eastep® Super Total RNA Extraction Kit) and the reverse transcription kit (GoScript™ Reverse Transcription System) were obtained from Promega Biotech Co., Ltd. (Shanghai, China). The fluorescence quantitative PCR kit (QuantiNova SYBR Green PCR Kit) was sourced from QIAGEN Shenzhen Co., Ltd. (Shenzhen, China). The centrifuge (model: Sigma 1-14k) was supplied by Sartorius, the PCR instrument (model: C1000 Touch™) was acquired from Bio-Rad (Hercules, CA, USA), and the fluorescence quantitative PCR instrument (model: Rotor-Gene Q) was provided by QIAGEN (Hilden, Germany).

Experimental groups and feeding management

The lambs were randomly assigned to four equal-sized groups: control group, low-dose betaine group (BET-L; 1 g/day per lamb), medium-dose group (BET-M; 3 g/day per lamb), and high-dose group (BET-H; 5 g/day per lamb). The lambs were raised in individual pens. Feeding was conducted twice daily, once in the morning and once in the evening, with betaine pre-mixed into the concentrated feed for the test groups. Initially, the lambs were provided with home-made concentrated pellet feed. After full consumption of the pellet feed, a mixed diet consisting of corn, whole corn silage, alfalfa, and peanut vine was provided. Feed and water were available *ad libitum*. The trial consisted of a 15-day preliminary adaptation period followed by a 153-day formal feeding period.

Determination of growth performance and blood biochemical indices

At the beginning and end of the trial, the lambs were weighed on an empty stomach to obtain the initial body weight (IBW) and final body weight (FBW), while the daily feed offered and the residual feed were recorded. The data collected above were used to calculate dry matter intake (DMI), average daily gain (ADG), and feed-to-gain ratio (F/G). The formulas used were as follows:

ADG (g/d) = (FBW – IBW) / number of trial days;

DMI (g/d) = total dry matter feed consumed / number of trial days;

F/G = DMI / ADG.

Blood samples (5 ml) were collected from the jugular vein of fasting lambs in the early morning at the end of the trial. The samples were centrifuged at 450 *g* for 10 min to separate plasma. The College of Veterinary Medicine, Shanxi Agricultural University was commissioned to analyse plasma cholesterol (CHO), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), urea nitrogen (BUN), and total protein (TP) contents. These measurements were taken to evaluate the effects of betaine on the physiological status of finishing Small-tailed Han sheep at the time of slaughter.

Determination of slaughter performance index and nutrient content in m. longissimus dorsi

Following the feeding trial, live weight was measured after a 24-h fasting period and 2 h of water deprivation. They were then sacrificed via venous exsanguination, followed by removal of the skin, head, limbs, and internal organs, while the kidneys and surrounding fat were retained. After resting for 30 min, the carcass was weighed and the dressing percentage was calculated.

A sample from the middle section of the longissimus dorsi muscle on the left side of the dorsal region was collected, rapidly frozen in dry ice, and sent to the Institute of Animal Science, Chinese Academy of Agricultural Sciences, for analysis of protein, fat, and water content.

Determination of key gene expression level in RNA extraction and lipid metabolic pathway of m. longissimus dorsi

A small tissue sample from the middle section of the longissimus dorsi muscle was excised using sterilized scissors, rapidly frozen in liquid nitrogen, and stored in an ultra-low-temperature freezer. Samples for gene expression analysis were selected based on the statistical significance of the muscle fat content data. Specifically, the control group and BET-H, which showed a significant difference in the muscle fat content ($P < 0.05$), were chosen to represent contrasting fat deposition phenotypes for mechanistic investigation. The tissue samples were ground into powder in liquid nitrogen using a mortar. Total RNA was extracted following the instructions of the total RNA extraction kit, and the RNA concentrations were adjusted to 100 ng/µl. The cDNA was synthesized from RNA of uniform concentration through reverse transcription, according to the protocol provided by the reverse transcription kit. The qRT-PCR system was set up following the guidelines of the fluorescence quantitative PCR kit, with each sample tested in triplicate.

The amplification process included pre-denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 10 s and annealing/extension at 60 °C for 20 s. Detailed primer sequences are presented in Table 2. The relative expression levels of target genes were calculated using the $2^{-\Delta\Delta CT}$ method.

Table 2. Primers for fluorescence quantitative PCR.

Gene	Primer sequence	GenBank accession No.	Product length (bp)
PPARα	F 5'-GATGTCCCATAACGCGATTC-3'		
	R 5'-GGCCTTGACCTTGTTCATGT-3'	XM_027968213.2	181
H-FABP	F 5'-GAGACCACGGCAGATGACAG-3'		
	R 5'-CCCGTCAACTATTTCCCGCA-3'	NM_001267884.2	120
HSL	F 5'- GGGTCATTGCCGACTTCCTA-3'		
	R 5'-GTCTCGTTGCGTTTGTAGTGC-3'	NM_001128154.1	160
FAS	F 5'-CTTAACAGCACGTCCCCCAT-3'		
	R 5'- CTCCTCGGGCTTGTCTTGTT-3'	XM_027974304.3	150
LPL	F 5'- AGACTCGTTCTCAGATGCCTT-3'		
	R 5'- CTCTCAGCCACAGTGCCAT-3'	NM_001009394.1	127
GAPDH	F 5'-GTCCGTTGTGGATCTGACCT-3'		
	R 5'-GGAGACAACCTGGTCCTCAG-3'	NM_001190390.1	130

PPARα - peroxisome proliferator-activated receptor α; H-FABP - heart fatty acid binding protein; HSL - hormone-sensitive triglyceride lipase; FAS - fatty acid synthase; LPL - lipoprotein lipase; GAPDH - glyceraldehyde phosphate dehydrogenase; F - forward primer; R - reverse primer

Statistical analysis

The test data were organized using Microsoft Excel and expressed as mean ± standard deviation. Statistical analysis was conducted using one-way ANOVA and Duncan's multiple comparison test in SPSS 18.0. Column charts were generated using GraphPad Prism 8. A value of $P < 0.05$ indicated significant differences.

Results

Effect of betaine on growth performance and blood indices in fattening lambs

As indicated in Table 3, dietary supplementation of different concentrations of betaine had no significant effect on the ADG, F/G, or DMI of fattening small-tailed Han sheep compared to the CON group ($P > 0.05$).

As presented in Table 4, supplementation of different concentrations of betaine had no significant effect on the CHO, TG, LDL, or HDL content of small-tailed Han sheep compared to the CON group ($P > 0.05$). Additionally, no significant effects of betaine supplementation were observed on BUN or TP contents ($P > 0.05$).

Effect of betaine on slaughter performance and nutrients in m. longissimus dorsi

As presented in Table 5, supplementation with various betaine concentrations had no significant effect on the pre-slaughter body weight of fattening lambs among the groups ($P > 0.05$). Similarly, no significant differences were observed in carcass weight or dressing percentage between the groups ($P > 0.05$). Analysis of the longissimus dorsi muscle tissue revealed no significant differences in crude protein and water content between the control group and the betaine-supplemented groups ($P > 0.05$). However, the BET-H group exhibited significantly higher fat content in the muscles compared to the other three groups ($P < 0.05$).

Table 3. Effect of betaine on growth performance of fattening lambs.

Item	Group			
	Control	BET-L	BET-M	BET-H
IBW/kg	15.60 ± 2.85	15.45 ± 2.46	15.63 ± 2.92	15.44 ± 2.83
FBW/kg	53.77 ± 4.65	54.22 ± 5.87	54.53 ± 6.31	54.48 ± 5.69
ADG (g/d)	226.76 ± 22.97	233.42 ± 20.92	238.63 ± 25.25	244.71 ± 23.82
DMI (g/d)	1057.32 ± 59.86	1070.64 ± 80.08	1051.48 ± 65.37	1129.91 ± 66.85
F/G	4.55 ± 0.78	4.57 ± 0.57	4.67 ± 0.91	4.68 ± 0.67

IBW - initial body weight; FBW - final body weight; ADG - average daily gain; DMI - dry matter intake; F/G - feed-to-gain ratio; BET-L - low-dose betaine group; BET-M - medium-dose group; BET-H - high-dose group

Table 4. Effect of betaine on blood indices of fattening lambs.

Item	Group			
	Control	BET-L	BET-M	BET-H
CHO (mmol/l)	2.15 ± 0.91	1.84 ± 0.25	2.07 ± 0.67	2.00 ± 0.49
TG (mmol/l)	0.69 ± 0.07	0.58 ± 0.04	0.66 ± 0.06	0.57 ± 0.07
LDL (mmol/l)	1.51 ± 0.67	0.95 ± 0.53	1.41 ± 0.39	1.31 ± 0.58
HDL (mmol/l)	1.28 ± 0.17	1.19 ± 0.13	1.25 ± 0.11	1.21 ± 0.18
BUN (mmol/l)	5.60 ± 0.12	5.91 ± 0.62	5.50 ± 0.19	5.41 ± 0.22
TP (g/l)	72.12 ± 3.41	71.20 ± 3.18	71.45 ± 3.27	69.95 ± 3.31

CHO - cholesterol; TG - triglyceride; LDL - low density lipoprotein; HDL - high density lipoprotein; BUN - urea nitrogen; TP - total protein; BET-L - low-dose betaine group; BET-M - medium-dose group; BET-H - high-dose group

Table 5. Effect of betaine on slaughter performance and m. longissimus dorsi nutrients of fattening lambs.

Item	Group			
	Control	BET-L	BET-M	BET-H
Body weight before slaughter (kg)	53.77 ± 4.65	54.22 ± 5.87	54.53 ± 6.31	54.48 ± 5.69
Carcass weight (kg)	28.72 ± 2.43	28.36 ± 2.16	28.71 ± 3.02	28.49 ± 2.87
Dressing percentage (%)	53.41 ± 2.31	52.30 ± 2.37	52.64 ± 2.46	52.29 ± 2.29
Crude protein (g/100 g)	19.77 ± 0.78	20.53 ± 0.97	20.34 ± 0.67	20.43 ± 0.94
Fat (g/100 g)	3.42 ± 1.04	3.71 ± 1.04	3.68 ± 1.12	4.27 ± 1.08[a]
Water (%)	72.72 ± 0.62	72.53 ± 0.70	72.68 ± 0.87	72.53 ± 0.63

BET-L - low-dose betaine group; BET-M - medium-dose group; BET-H - high-dose group

[a] The value differs at $P < 0.05$

Effect of betaine on key gene expression in lipid metabolic pathway

The gene expression results for the BET-H and control groups, which exhibited the greatest difference in fat content within the longissimus dorsi muscle, are presented in Fig. 1. The BET-H group demonstrated increased mRNA expression of the heart-type fatty acid-binding protein (*H-FABP*), lipoprotein lipase (*LPL*), and fatty acid synthase (*FAS*) genes in the longissimus dorsi muscle, with a significant increase in LPL expression compared to the control group ($P < 0.05$). Conversely, the mRNA expression levels of the peroxisome proliferator-activated receptor alpha (*PPARα*) and lipase E, hormone-sensitive type (*LIPE*) genes were not significant among the groups ($P > 0.05$).

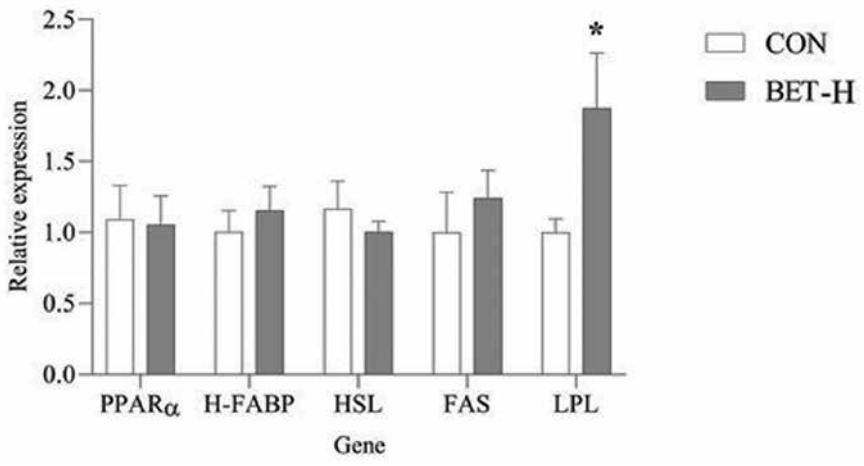

Fig. 1. Effect of betaine on gene expression in the fat metabolism pathway of fattening lambs

PPARα - peroxisome proliferator-activated receptor; H-FABP - heart fatty acid binding protein; HSL - hormone-sensitive triglyceride lipase; FAS - fatty acid synthase; LPL - lipoprotein lipase; CON - control group; BET-H - high-dose group; * reflects significant difference ($P < 0.05$).

Discussion

Effect of betaine on growth performance and blood biochemical indices

Betaine is recognized as a multifunctional feed additive. Studies have demonstrated its use as a feed attractant to enhance intake in animals, particularly in fish (Lim et al. 2015). Dong et al. (2019) reported that betaine supplementation increased feed intake in Hu lambs, whereas Jiang (2017) reached a different conclusion under similar conditions. In the present study, no significant improvement in feed intake was observed following the addition of varying concentrations of betaine to the diet of fattening lambs, which aligns with the findings of Jiang (2017).

As a highly effective methyl donor, betaine participates in amino acid metabolism and promotes protein synthesis, thereby influencing the growth performance of animals (Cheng et al. 2021). Wang et al. (2020a) reported that betaine supplementation increased the ADG in piglets, while Lakhani et al. (2020) observed a similar effect in calves. However, Fernández et al. (2009) found that betaine had minimal impact on the growth performance of lambs. In subsequent research, they noted that betaine supplementation effectively promoted ruminant growth only when methionine concentrations in the feed were inadequate (Fernández et al. 2009).

In this study, betaine supplementation did not significantly improve the ADG of fattening lambs, which is consistent with the findings of Fernández et al. (2009). However, further research is required to determine if this outcome is influenced by the methionine content of the feed.

Blood biochemical analysis is a crucial method for monitoring metabolic activities in living organisms. The assessment of biochemical indicators in this study provided insights into protein and fat metabolism. Cui (2015) reported that the addition of rumen-protected betaine to the diet of Hu lambs significantly decreased serum concentrations of CHO, TG, LDL, and UN, while significantly increasing TP contents. These findings indicated that betaine could promote protein synthesis while reducing fat deposition. However, Cai et al. (2021) did not observe significant changes in these biochemical indices under similar experimental conditions, which was consistent with the findings of this study.

Effect of betaine on slaughter performance and nutrients in m. longissimus dorsi

Slaughter performance serves as an important indicator of growth efficiency in livestock and poultry. Yang et al. (2022) reported that betaine supplementation in Xueshan chickens improved dressing percentage. However, research by Li et al. (2015) on fattening sheep revealed that various concentrations of dietary betaine did not significantly affect the dressing percentage of fattening lambs, a finding consistent with the results of the present study.

Betaine regulates fat metabolism and distribution through multiple mechanisms. In HepG2 cells, it influences RNA methylation, inhibits fatty acid synthesis, and promotes fatty acid oxidation, thereby reducing liver fat deposition (Zhang et al. 2019). In mice that were fed a high-fat diet, betaine prevented lipid accumulation in myocytes by altering fatty acid composition and inhibiting fatty acid synthesis (Du et al. 2018). In lambs, it reduced belly fat content through the mTOR signalling pathway while increasing fat content in lamb meat (Dong et al. 2020).

In this study, the high-dose betaine group exhibited increased fat content in the longissimus dorsi muscle. This increase in intramuscular fat enhances meat tenderness (Ouali et al. 2013) and juiciness (Wood et al. 2008), thereby improving overall meat quality. However, the precise mechanisms underlying this regulatory effect require further investigation.

Effect of betaine on key gene expression in lipid metabolic pathway

Fat biosynthesis is regulated by various synthases and transcription factors that play essential roles in lipid metabolism. The most commonly cited and rate-limiting enzymes within the specific pathways most relevant to ovine adipose tissue deposition include FAS, hormone-sensitive lipase (HSL), and LPL (Xu et al. 2018). Additionally, regulatory factors such as H-FABP (Lang et al. 2017) and peroxisome proliferator-activated receptor alpha (*PPARα*) also contribute to lipid metabolism (Guo et al. 2022). Current research on the role of betaine in fat metabolism and its associated gene expression has primarily focused on liver fat metabolism. Yao et al. (2021) demonstrated that betaine supplementation significantly reduced liver fat content in Tibetan chickens, accompanied by decreased mRNA expression of *FAS* and acetyl-CoA carboxylase (*ACC*) genes and increased *PPARα* mRNA expression. Similarly, Wang et al. (2020b) found that betaine reduced liver fat content in mice that were fed a high-fat diet, along with significant downregulation of *LPL* and *FAS*, which are involved in fat synthesis.

In muscle tissue, betaine has been shown to promote fat accumulation, although the precise molecular mechanisms remain to be elucidated. Wu et al. (2018) reported that adding 10 mM of betaine to a C2C12 muscle cell culture medium enhanced differentiation into adipocytes, accompanied by upregulation of *PPARγ* and its downstream genes, *FAS* and *LPL*. These findings suggest that betaine activates the ERK/*PPARγ* signalling pathway, thereby promoting fat deposition in muscle cells. Albuquerque et al. (2017) observed that betaine significantly increased the mRNA expression of *LPL* and *H-FABP* in the longissimus dorsi muscle while reducing HSL expression.

In the present study, the mRNA expression level of the LPL gene in the longissimus dorsi muscle of lambs in the BET-H group was significantly higher than that in the control group. These findings align with previous studies, indicating that betaine may enhance intramuscular fat accumulation by promoting fat synthesis.

Emerging research suggests that betaine regulates gene expression through DNA methylation (Chen et al. 2019). Yang et al. (2021) demonstrated that betaine inhibited hepatic lipid accumulation and increased total DNA methylation in geese. Therefore, further research is needed to investigate whether betaine regulates *LPL* gene expression through DNA methylation and its subsequent effects on fat metabolism.

Study limitations and future directions

This study had several limitations. The long-term effects of betaine supplementation were not assessed, and its interactions with other nutrients were not extensively analysed. Due to the lack of blood biochemical data at the initiation of feeding, temporal changes in these indicators could not be assessed in the present study. Additionally, the tissue-specific distribution of fat was not fully analysed, and other key meat quality indicators were not extensively explored. The metabolic pathway of betaine and its underlying mechanism of action were not comprehensively examined. Moreover, the sample size and repeatability of the experiment require improvement to enhance the robustness of the findings.

Future studies should focus on optimizing dosage strategies and conducting long-term feeding experiments. Further investigation into nutrient interactions, a comprehensive analysis of fat distribution, and a detailed assessment of meat quality indicators are recommended. Additionally, a more in-depth exploration of the metabolic pathways and molecular mechanisms of betaine should be carried out using multi-omics approaches. These improvements could enhance the reliability and applicability of research findings, providing a stronger scientific foundation for the use of betaine in sheep production.

In conclusion, supplementing the diet of fattening Small-tailed Han sheep with 5 g/day per head of betaine did not affect meat production performance but promoted intramuscular

fat deposition in the longissimus dorsi muscle. Further analysis of the expression of key enzymes involved in intramuscular fat synthesis revealed a significant upregulation of LPL mRNA expression. This suggests that betaine may influence body fat distribution in fattening male lambs of Small-tailed Han sheep by regulating lipid metabolism-related genes.

Conflict of interest

The authors declare that they have no competing interests.

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