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Corresponding author: Qing-Chang Ren; Email: Renqc@ahstu.edu.cn

Effects of supplementing tributyrin on serum biochemical indices and meat quality characteristics of longissimus thoracis et lumborum of weaned Small-Tailed Han lambs

Ya-Xin Wang[1], Xue-Er Wang[2], Zhi-Wei Li[1], Ran An[1], Qing-Chang Ren[1,3] and Jian-Zhuang Tan[4]

[1]College of Animal Science, Anhui Science and Technology University, Fengyang, P R China; [2]College of Animal Science and Technology, Tarim University, Alae, P R China; [3]Anhui Province Key Laboratory of Animal Nutritional Regulation and Health, Anhui Science and Technology University, Fengyang, P R China and [4]Swine Nutrition Institute, Techbank Food Co. LTD., Hefei, P R China

Abstract

This experiment aimed to investigate the impacts of tributyrin (TB) dietary supplementation on serum biochemical indices and meat quality characteristics of longissimus thoracis et lumborum (LTL) muscle of lambs after weaning. Thirty healthy Small-Tailed Han female lambs (27.5 ± 4.1 kg; mean ± standard deviation) were randomly assigned to five treatments: basal diet (1) without TB, (2) with 0.5 g/kg TB, (3) with 1.0 g/kg TB, (4) with 2.0 g/kg TB or (5) with 4.0 g/kg TB. Each treatment consisted of six lambs, and the lambs were weaned on d 90 and were raised until d 165. Results showed that supplementing TB significantly promoted serum immunoglobulin concentrations of lambs such as immunoglobulins G, A and M. Besides, TB significantly increased muscle ether extract content, intermuscular fat length, pH value and redness but decreased lightness, drip loss and shear force. In addition, TB significantly elevated inosine-5′-phosphate content and upregulated the relative expressions of genes related to lipid metabolism such as *SREBP-1C, SCD, PPARγ, FAS* and *LPL*. The mostly important, TB significantly enhanced essential amino acids (EAAs) and conjugated linoleic acids contents of the LTL muscle, despite it decreased total unsaturated fatty acids level. In conclusion, supplementing TB not only could promote the healthy status of weaned lambs via promoting serum immunity but also may improve nutritional quality of LTL muscle by improving EAA and conjugated linoleic acid contents.

Introduction

It has been long known that the mutton acceptability of consumer is influenced by many attributes such as regional or culture factors and its nutritional quality (Zhang et al. 2020). During the recent years, the nutritional value becomes an increasingly important factor influencing consumer preference for sheep mutton, which depends on fatty acid (FA) composition, amino acids (AAs) content, trace minerals and vitamins and affects changes to benefit for human health (Scollan et al. 2017). Therefore, targeted nutritional strategies are needed to improve mutton FA composition and AA content to meet the increasing demands of consumers. However, the presence of the rumen makes mutton FA composition more difficult to manipulate in comparison to pig. First, a large-scale proportion of unsaturated fatty acids (UFAs) can be biohydrogenated by ruminal bacteria so that the FAs in sheep meat are more saturated than those in pigs when diet lipid enters the rumen and undergoes the microbial biohydrogenation (Wood et al. 1999). Besides, the mutton quality might also be affected by the synthesis level of microbial crude protein (MCP) in the rumen, which plays a key nutrition role in meeting the qualitative AA requirements of growing lambs (Nolte 2006). In addition, the biosynthesis of FA in ruminant meat is also influenced by cleaving effects at tissue level at the presence of lipoprotein lipase enzyme activity, which is often regulated by the expression of genes such as *SREBP-1C, SCD, PPARγ, FAS* and *LPL* (Oliveira et al. 2014). In these regards, by changing rumen microbial activity and population and the expression of genes related to lipid metabolism is possible to modify both FA and AA contents in mutton.

Recently, tributyrin (TB) has been attracted much attention due to its benefits on stimulating colonization of gastrointestinal microflora in ruminant animals. Liu et al. (2022) reported that supplementing TB in milk replacer of dairy calves before weaning could effectively stimulate the relative abundances of volatile fatty acids (VFAs)-producing bacteria such as *Ruminococcaceae, Lachnospiraceae, Prevotella* and *Rikenellaceae* in small intestine.

Currently, Li et al. (2023) reported that addition of TB into diet of lambs after weaning could also significantly increase the relative abundances of VFAs-producing bacteria such as *Clostridium, Butyrivibrio, Streptococcus, Prevotella, Ruminobacter* and *Fibrobacter* in the rumen. Besides, supplementing TB has been shown to enhance MCP synthesis in the rumen of adult sheep and to increase both *in vitro* and *in vivo* VFA's formation (Ren et al. 2018a, 2018b, 2018c; Song et al. 2020). Given the above effects of TB on modifying bacteria population and metabolism in ruminant gastrointestinal tract particularly in the rumen, TB addition into feed was hypothesized to have the potential to modify the FA level as well as AA content in sheep meat. Thus, this test was carried out to assess the impacts of supplementing TB on serum biochemical indices and meat quality characteristics of longissimus thoracis et lumborum (LTL) muscle of weaned Small-Tailed Han lambs.

Materials and methods

The statement of animal welfare

Before starting the present experiment, the procedures involved were approved by the Animal Ethics Committee of Anhui Science and Technology University (approval no. 2023007) based on the principles of animal welfare such as reduction, replacement and refinement. Since the current experiment was carried out from June to August in 2023, all efforts such as physical cooling and other measures were taken to minimize the harm of heat stress on the used lambs.

Lambs and the experimental design

In order to well facilitate management and meet the local consuming habit of low-flavor mutton, 30 healthy female lambs of Small-Tailed Han sheep were selected as the experimental animals. The lambs with a mean live body weight of 27.5 $\pm$ 4.1 kg (mean $\pm$ standard deviation) were randomly assigned to five treatments: basal diet (1) without TB, (2) with 0.5 g/kg TB, (3) with 1.0 g/kg TB, (4) with 2.0 g/kg TB, or (5) with 4.0 g/kg TB. Each treatment consisted of six lambs, and the lambs were weaned on d 90. After weaning, each lamb was individually raised in a metabolism cage (2.25 m^2) until d 165. Every day, each lamb had *ad libitum* access to water and the basal diet, which was provided as a total mixed ration (Table 1) at 07:00 and 19:00. According to the dry matter (DM) content of the basal diet, each treatment lambs received TB dose ranged from 0 to 4.0 g/kg of DM. The used TB was purchased from Perstorp (Shanghai) Chemical Products Trading Co., Ltd., and the selected dosages of TB were consistent with Wang et al. (2024), who added TB into feed of weaned lambs with varied dosages from 0 to 4.0 g/kg of DM.

Sample collection

Based on the final meal of local slaughtering culture, the used lambs were sacrificed 3 h after feeding in the morning. An hour before slaughtering, 10 mL $EDTAK_2$ vacuum serum sampling tubes purchased from Guangzhou Kangcai Medical Equipment Manufacturing Co. Ltd. (Guangzhou, Guangdong province, China) were used to sample blood from the jugular vein of each lamb. The blood samples were left 30 min and centrifuged at 4°C with 1,500 × g for 10 min, then the upper serum was obtained and stored at −20°C until the serum biochemical indices were analyzed.

Table 1. Ingredient and nutrient composition (g/kg, as DM basis) and amino acid and fatty acid contents of the total mixed ration fed for weaned Small-Tailed Han female lambs

Items	Content
Ingredients (g/kg, as DM basis)	
Maize	250
Soybean meal	110
Ensiled total corn stover	350
Peanut straw	200
Garlic by-products	50.0
Premix[1]	40.0
Nutrients (g/kg, as DM basis)	
Metabolizable energy[2], MJ/kg	12.3
Crude protein	181
Ether extract	31.0
NDF	373
ADF	242
Non-fiber carbohydrate[3]	341
Ash	74.0
Ca	7.00
Total P	4.00
Amino acids (g/100 g DM)	
Aspartic acid	0.57
Threonine	0.27
Serine	0.29
Glutamic acid	1.08
Proline	0.43
Glycine	0.31
Alanine	0.45
Cystine	0.13
Valine	0.44
Methionine	0.17
Isoleucine	0.33
Leucine	0.59
Tyrosine	0.31
Phenylalanine	0.24
Histidine	0.14
Lysine	0.36
Arginine	0.28
Fatty acids (mg/100 g DM)	
C6:0	3.38
C14:0	7.68
C16:0	284
C16:1n9c	4.99
C18:0	56.3

(*Continued*)

Table 1. (*Continued.*)

Items	Content
C18:1n9c	272
C18:2n6c	537
C18:3n3	51.5
C20:0	10.4
C22:0	8.60
C24:0	8.63

DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber.
[1]The premix fed to weaned lambs in this experiment consisted of vitamin A, 15.4 × 10^4 IU/kg; vitamin D_3, 9.4 × 10^4 IU/kg; vitamin E, 33.8 v 10^4 IU/kg; I, 0.12 g/kg; Cu, 0.28 g/kg; Fe, 2.24 g/kg; Mn, 1.74 g/kg; Zn, 1.37 g/kg; Se, 0.06 g/kg, Co, 16.8 mg/kg; lysine, 0.05 g/kg; methionine, 0.05 g/kg.
[2]Metabolizable energy was calculated according to NRC (2001).
[3]Non-fiber carbohydrate was calculated as follows: NFC (g/kg) = 1000 – NDF – crude protein – ether extract – ash.

In this study, the portion of the LTL muscle included both longissimus thoracis and longissimus lumborum. Meanwhile, the LTL was defined as the muscle in the triangular region formulated by processus spinous of both thoracic vertebra and lumbar vertebra, the upper ribs and processus transverse of lumbar vertebra. After slaughter, carcass was cut up and both left and right sides of the LTL muscle were excised from the 6 th to the 13th vertebra under aseptic conditions according to the method of NY/T 1564-2007 (AIS 2007). About 5 g of the LTL muscle without fascia was immediately frozen using liquid nitrogen for 60 s, then it was stored at −80°C until RNA was extracted. In addition, muscle pieces of 10 g each were vacuum-packed and stored at −20°C to extract intramuscular fat, to analyze AAs, to determinate nucleotides content and the muscle nutrients, respectively. The left LTL muscle was used to determine the muscle pH value, color (e.g., lightness, redness and yellowness), water holding capacity (e.g., drip loss and cooking loss) and meat texture such as hardness and chewiness.

Determination of the serum biochemical indices

Aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase, total protein, globe protein, albumin, glucose, urea, cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium, magnesium, phosphorus and creatinine concentrations in the serum of each lamb were analyzed by multipara metric auto-analyzer (ILab 650 type; Instrumentation Laboratory Company, Lexington, MA, USA). In addition, immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) (g/L) were determined using immunoglobulin ELISA kits (Shanghai Yubo Biotechnology Co. Ltd., Shanghai city, China). Each sample was determined in triplicate.

Chemical analysis of both muscle and feed

According to the methods of AOAC (2012), the nutrient content of basal diet such as DM, nitrogen, ether extract, crude ash including calcium and phosphorus was determined, while metabolizable energy was estimated based on NRC (2001). For fiber carbohydrates, neutral detergent fiber and acid detergent fiber were analyzed using α-thermoamylase (Van Soest et al. 1991). According to our previous methods (Wang et al. 2024), intermuscular fat length and width of the LTL muscle were determined using Znx-5 Eyepiece micrometer purchased from Dongguan Zhunna Optoelectronic Technology Co. Ltd. (Dongguan, Guangdong province, China), which was corrected with Type C 1 OLM 1/100 micrometer purchased from Beijing Jiangfengjia Strength Supplier (Beijing city, China) before determination.

Determinations of muscle pH, color, water holding capacity and texture

The muscle pH value of the LTL was determined after 15 min post mortem by insertion of a glass electrode HI8424 attached to portable meter with automatic temperature compensation (Beijing Hanna Instruments Science & Technology Co. Ltd., Beijing city, China), and the temperature compensation ranged from 0°C to 100°C. Before measurement, the pH meter was calibrated using both pH 4.00 and pH 6.86 standard buffer solutions prepared in a water bath at 25°C. Briefly, the pH meter was first corrected with 6.86 standard buffer, followed by 4.00 standard buffer. For each correction, the temperature compensation knob was also adjusted according to the actual measured temperature. At last, the correction was finished if the deviation did not exceed 0.02 pH unit during the determination of the pH values of the used standard buffers. Closed to the insertion point of the temperature probe, the reading pH value was automatically adjusted for carcass temperature. After waiting for about 40 s, the stable reading was recorded. For each side of the LTL muscle, the measurement was repeated in triplicate.

The fresh cutting surface of approximately 20 mm thick piece was bloomed at 4°C in the air for 20 min. Before measuring muscle color, the used CR-10 colorimeter (Spectrophotometer, Minolta, Tokyo, Japan) with illuminant D65, 10° viewing angle geometry, 8-mm-diameter measurement area was calibrated with a pure white plastic sheet (PVC material). The muscle color was determined in triplicate perpendicular to the muscle's surface and recorded in terms lightness, redness and yellowness. In this study, meat texture profiling was described with terms of hardness, cohesiveness, springiness, gumminess and chewiness. According to Ren et al. (2019), the internal temperature of each LTL muscle was first cooked to 75°C, then it was cooled to room temperature before measurement of meat texture with a Model A-XT2 texture analyzer (Stable Micro Systems, Surrey, UK). In this experiment, three LTL samples from each lamb were used to determine cooking loss after color measurement. The determination was performed in a cooking batch and each sample was weighted about 30 ± 3 g (W1). During the cooking, a digital thermometer (Ningbo Kaitai Electric Appliance Industry Co. Ltd., model WT7-1, Ningbo, Zhejiang province, China) was inserted into the center of each sample to measure the core temperature until it reached to 70°C. After cooling, drying and weighing (W2), cooking loss (g/100 g) of the LTL was calculated as 100 × (W1 – W2)/W1. Meanwhile, each LTL sample of 10 ± 1 g was suspended for 24 h at 4°C in a plastic bag to determine drip loss in triplicate. Shear force value (N) was determined using a Warner–Bratzler shearing device fitted to a C-LM3B digital muscle tenderness meter purchased from Northeast Agricultural University (Harbin, Heilongjiang province, China). In the present study, the shear force was expressed as the average force required to shear through the LTL core.

Determination of AAs, FAs and nucleotides

In the present test, AAs content in the LTL sample of each lamb was determined using a UPLC-Orbitrap-MS system (UPLC, Vanquish;

MS, QE) according to the method of GB 5009.124 (NHFPC 2016a), while FAs content was measured according to the methods of GB 5009.168-2016 (NHFPC 2016b) with Agilent HP6890 (Agilent Technologies, California, USA). Nucleotides such as inosine-5′-phosphate (5′-IMP) and guanosine-5′-monophosphate (5′-GMP) were analyzed according to the methods of GB 5413.40-2016 (NHFPC 2016c) using Agilent 7890B/7000C (Agilent Technologies, CA, USA), which equipped with a shim-pack C18 column (2.1 mm × 100 mm, 1.8 μm particle size). The detailed determination procedures of AAs, FAs and nucleotides could respectively be obtained from our previous reports in 2.6. Analysis of AAs Content, 2.7. Analysis of FAs Composition and 2.8. Determination of Nucleotides Content (Wang et al. 2024).

Measurement of the relative expression of genes related to lipid metabolism

In the current experiment, genes related to lipid metabolism such as *SREBP-1C, SCD, PPARγ, ACC, FAS* and *LPL* were measured using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). Briefly, about 0.5 g sample of the LTL was used to extract RNA, and the RNA was detected by 10 g/L agarose gel electrophoresis, and the concentration of RNA was diluted to 500 ng/μL, followed by cDNA synthesis using PrimeScript 1st Strand cDNA Synthesis Kit (TakaRa Bio, Shiga, Japan). RT-qPCR was performed on cDNA with SYBR dye and Light Cycler 480 system (Roche Diagnostics, Basel, Switzerland), and primers of *SREBP-1C* (F: 5′-CTCCGACACCACCAGCA TCAAC-3′; R: 5′-GCAGCCCATTCA TCAGCCAGAC-3′), *SCD* (F: 5′-GAGTACCGCTGGCACAT CAA-3′; R: 5′-CTAA GACGGCAGCCTTGGAT-3′), *PPARγ* (F: 5′-CACCACCGTT GACTTCTCCA-3′; R: 5′-TGATCACACGTTCCACCTC GTC-3′), *ACC* (F: 5′-ATGTTTCGGCAGTCCCTG AT-3′; R: 5′-TGTGGACCAGCTGACCTTGA-3′), *FAS* (F: 5′-GTGTGGT ACAGCC CCTCAAG-3′; R: 5′-ACGCACCTGAATGACC ACTT-3′) and *LPL* (F: 5′-TCATCG TGGTGGACTGGCT-3′; R: 5′-CATCCGCCATCCAGTTCATA-3′) were selected, which was used by Wang et al. (2022) as the primers to determine the relative expressions of the present target genes in longissimus dorsi of Small-Tailed Han sheep. In the present study, both *β-actin* and *GAPDH* genes were used as dual internal standard for normalizing transcript abundance of mRNA expression, and the relative expressions of the *SREBP-1C, SCD, PPARγ, ACC, FAS* and *LPL* were calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

The present data were analyzed using SAS 9.4 with PROC MIXED model. For measurement of feed nutrient including FA, treatment group was considered as experimental unit. For determinations of serum biochemical indices, muscle chemicals, pH, color, water holding capacity and texture, analysis of AAs, FAs and nucleotides contents as well as measurement of the relative expression of genes related to lipid metabolism, individual lamb was used as the experimental unit, and all samples were repeated in triplicate each time. The effects of dietary TB supplementation were evaluated using Contrast (0 vs TB), Linear and Quadratic effects. Meanwhile, the significant level of the comparison among treatments mean was conducted using Duncan's multiple range test. In the present experiment, the difference among treatments mean was considered significant at $P < 0.05$. The used PROC MIXED model including random and fixed effects as follows:

$$Y_{ij} = \mu + L_i + T_j + \varepsilon_{ij}$$

where Y_{ij} is the dependent variable, μ is the overall mean, L_i is the random effects of lambs (i = 6), T_j is the fixed effects of supplementing TB ($j = 0$, 0.5, 1.0, 2.0 and 4.0 g/kg DM) and ε_{ij} is the error term.

Results

Effects of supplementing TB on serum biochemical indices of weaned lambs

Table 2 shows that supplementing TB linearly decreased serum activity of lactate dehydrogenase ($P = 0.049$) and concentration of urine nitrogen ($P = 0.015$), but increased serum concentrations of IgG ($P < 0.001$), IgA ($P < 0.001$), IgM ($P < 0.001$), HDL ($P < 0.001$) and calcium ($P < 0.001$). In the current experiment, there were significant effects of TB on neither serum activities of AST and ALT nor on serum concentrations of total protein, globe protein and albumin, the ratio of albumin to globe protein, glucose, cholesterol, triglyceride, LDL, magnesium and creatinine.

Effects of supplementing TB on nutritional compositions of LTL muscle

Compared with lambs fed without TB, ether extracts in the LTL muscle of lambs fed with TB were increased by 12.6%, 21.7%, 9.64% and 8.04% ($P = 0.018$). Besides, muscle calcium contents were increased by 11.9%, 60.8%, 29.4% and 27.7% ($P = 0.044$), while the muscle phosphorus levels were enhanced by 30.4%, 56.1%, 51.5% and 32.7% ($P = 0.001$). Meanwhile, intermuscular fat length was also increased by 21.3%, 34.3%, 10.6% and 34.3% ($P = 0.023$) with increasing TB. As shown in Table 3, supplementing TB had no significant effects on muscle contents of DM, protein, ash and intermuscular fat width in the LTL muscle of weaned lambs.

Effects of TB on pH, color, water holding capacity and shear force of LTL muscle

With increasing TB supplementation, the LTL muscle pH values were increased by 5.00%, 4.84%, 3.71% and 5.48% ($P < 0.001$). Furthermore, the muscle redness was also increased by 4.00%, 26.4%, 25.6% and 12.0% ($P = 0.011$). But the muscle lightness in the LTL muscle of lambs fed with TB was decreased by 15.6%, 15.9%, 14.8% and 18.4% ($P < 0.001$). Besides, drip loss was decreased by 38.6%, 37.7%, 35.4% and 37.7% ($P < 0.001$), while cooking loss was reduced by 21.2%, 13.7%, 38.5% and 23.9% ($P < 0.001$). In addition, the muscle shear force was decreased by 37.2%, 29.5%, 23.0% and 18.3% ($P < 0.001$). As shown in Table 4, supplementing TB had no significant impact on muscle yellowness.

Effects of supplementing TB on meat texture of LTL muscle of weaned lambs

As shown in Table 5, supplementing TB had negative effects on meat texture of the LTL muscle of weaned lambs. With increasing TB supplementation, the LTL hardness was linearly decreased by 3.24%, 5.95%, 8.11% and 11.4% ($P < 0.001$). Besides, cohesiveness was reduced by 7.87%, 6.74%, 10.1% and 13.5% ($P < 0.001$), springiness was decreased by 16.3%, 12.2%, 14.3% and 30.6%

Table 2. Effects of dietary supplementation with tributyrin on serum parameters of Small-Tailed Han lambs

Items	Tributyrin additions, g/kg DM basis					SEM	*P*-values		
	0	0.5	1.0	2.0	4.0		Contrast	Linear	Quadratic
Aspartate transaminase (U/L)	127	120	141	139	127	8.20	0.606	0.487	0.366
Alanine transaminase (U/L)	11.1	13.6	14.0	14.8	16.5	2.01	0.122	0.074	0.890
Lactate dehydrogenase (U/L)	659[a]	608[ab]	529[b]	571[ab]	570[ab]	33.0	0.022	0.049	0.274
Total protein (g/L)	57.1	60.2	66.3	58.6	60.8	2.32	0.108	0.435	0.047
Globe protein (g/L)	35.5	36.6	42.3	35.8	38.0	1.80	0.190	0.474	0.019
IgG (g/L)	3.21[b]	3.60[a]	3.71[a]	3.72[a]	3.71[a]	0.05	$<$0.001	$<$0.001	0.787
IgA (g/L)	9.20[c]	9.61[b]	9.80[ab]	10.0[a]	10.0[a]	0.09	$<$0.001	$<$0.001	0.387
IgM (g/L)	1.20[b]	1.51[a]	1.50[a]	1.61[a]	1.62[a]	0.03	$<$0.001	$<$0.001	0.433
Albumin (g/L)	21.6	23.5	24.0	22.8	22.8	0.89	0.232	0.798	0.201
The ratio of albumin to globe protein	0.61	0.64	0.57	0.64	0.60	0.08	0.739	0.710	0.175
Glucose (mmol/L)	1.50	1.51	1.13	1.52	1.80	0.37	0.873	0.603	0.455
Blood urine nitrogen (mmol/L)	8.20[a]	7.11[ab]	7.13[ab]	6.62[ab]	6.24[b]	0.55	0.032	0.015	0.624
Cholesterol (mmol/L)	1.21	1.40	1.51	1.53	1.41	0.10	0.105	0.445	0.856
Triglyceride (mmol/L)	0.19	0.20	0.21	0.20	0.22	0.02	0.254	0.289	0.892
Low-density lipoprotein (mmol/L)	1.31	1.40	1.31	1.41	1.33	0.05	0.812	0.339	0.121
High-density lipoprotein (mmol/L)	2.12[c]	2.33[b]	2.61[a]	2.63[a]	2.62[a]	0.06	$<$0.001	$<$0.001	0.070
Ca (mmol/L)	2.20[b]	2.31[b]	2.52[ab]	2.51[ab]	2.80[a]	0.12	0.019	$<$0.001	0.347
Mg (mmol/L)	0.69	0.72	0.86	0.80	0.81	0.06	0.107	0.157	0.112
P (mmol/L)	2.34[b]	2.60[ab]	2.72[a]	2.65[ab]	2.54[ab]	0.12	0.013	0.931	0.041
Creatinine (μmol/L)	59.1	57.6	63.2	53.4	51.5	4.15	0.556	0.134	0.239

DM = dry matter; SEM = standard error of the mean.
[a–c]Values within a row with no common superscripts differ significantly ($P < 0.05$).

Table 3. The effects of tributyrin on nutritional compositions of longissimus thoracis et lumborum of Small-Tailed Han lambs

Items	Tributyrin additions, g/kg DM basis					SEM	*P*-values		
	0	0.5	1.0	2.0	4.0		Contrast	Linear	Quadratic
DM (g/100 g)	25.2	25.7	26.5	25.0	24.3	1.05	0.883	0.438	0.491
Protein (g/100 g)	18.9	18.8	19.2	18.3	17.7	1.03	0.696	0.379	0.656
Ether extract (g/100 g)	5.60[b]	6.31[ab]	6.82[a]	6.14[b]	6.05[b]	0.22	0.018	0.594	0.016
Ash (mg/100 g)	573	581	565	654	525	83.3	0.927	0.930	0.520
Ca (mg/100 g)	4.11[b]	4.60[b]	6.61[a]	5.32[ab]	5.25[ab]	0.57	0.044	0.113	0.033
P (mg/100 g)	43.3[b]	56.5[ab]	67.6[a]	65.6[a]	57.5[ab]	4.67	0.001	0.017	0.646
Intermuscular fat length (μm)	30.0[b]	36.4[ab]	40.3[a]	33.2[ab]	40.3[a]	2.78	0.023	0.049	0.4156
Intermuscular fat width (μm)	10.2	11.9	13.3	11.8	10.4	1.34	0.290	0.964	0.632

DM = dry matter; SEM = standard error of the mean.
[a, b]Values within a row with no common superscripts differ significantly ($P < 0.05$).

($P < 0.001$), gumminess was decreased by 11.1%, 12.3%, 17.85% and 23.9% ($P < 0.001$), while chewiness was reduced by 23.6%, 23.1%, 29.2% and 47.1% ($P < 0.001$), respectively.

Effects of supplementing TB on AAs composition in LTL muscle of weaned lambs

As shown in Table 6, supplementing TB linearly increased muscle content of essential amino acids (EAAs) ($P = 0.002$), including methionine ($P < 0.001$), isoleucine ($P < 0.001$), leucine ($P = 0.010$), phenylalanine ($P = 0.050$) and lysine ($P = 0.006$). Besides, TB linearly promoted the LTL content of nonessential amino acids (NEAAs) ($P < 0.001$) including proline ($P < 0.001$), glutamic acid ($P < 0.001$), glycine ($P = 0.002$), histidine ($P < 0.001$), alanine ($P < 0.001$), arginine ($P < 0.001$), aspartic acid ($P < 0.001$), cystine ($P = 0.001$) and tyrosine ($P = 0.014$). Thus, TB increased the determined εAAs ($P < 0.001$), branched-chain AAs ($P < 0.001$), umami ($P < 0.001$) and sweet AAs

Table 4. The effects of tributyrin on pH, color, water holding capacity and shear force in longissimus thoracis et lumborum of Small-Tailed Han lambs

	Tributyrin additions, g/kg DM basis						*P*-values		
Items	0	0.5	1.0	2.0	4.0	SEM	Contrast	Linear	Quadratic
pH[1]	6.20[b]	6.51[a]	6.50[a]	6.43[a]	6.54[a]	0.04	<0.001	<0.001	0.979
Lightness	35.8[a]	30.2[b]	30.1[b]	30.5[b]	29.2[b]	1.27	<0.001	0.002	0.793
Redness	12.5[b]	13.0[b]	15.8[a]	15.7[a]	14.0[ab]	0.70	0.011	0.016	0.313
Yellowness	2.51	2.32	2.63	2.94	2.75	0.23	0.815	0.275	0.911
Drip loss $_{24h}$ (g/100 g)	8.52[a]	5.23[b]	5.31[b]	5.50[b]	5.31[b]	0.70	<0.001	0.007	0.679
Cooking loss (g/100 g)	33.5[a]	26.4[b]	28.9[ab]	20.6[c]	25.5[bc]	1.81	<0.001	<0.001	0.004
Shear force (N)	33.9[a]	21.3[c]	23.9[bc]	26.1[bc]	27.7[b]	2.00	<0.001	0.244	0.036

DM = dry matter; SEM = standard error of the mean.
[a-c]Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Muscle pH value was measured at 15 min post mortem.

Table 5. The effects of tributyrin on texture of cooked longissimus thoracis et lumborum of Small-Tailed Han lambs

	Tributyrin additions, g/kg DM basis						*P*-values		
Items	0	0.5	1.0	2.0	4.0	SEM	Contrast	Linear	Quadratic
Hardness (g)	185[a]	179[b]	174[bc]	170[cd]	164[d]	2.03	<0.001	<0.001	0.980
Cohesiveness	0.89[a]	0.82[b]	0.83[b]	0.80[b]	0.77[b]	0.020	<0.001	<0.001	0.334
Springiness	0.49[a]	0.41[b]	0.43[ab]	0.42[ab]	0.34[c]	0.023	0.001	<0.001	0.781
Gumminess (g)	41.5[a]	36.9[b]	36.4[b]	34.1[bc]	31.6[c]	1.02	<0.001	<0.001	0.399
Chewiness (g)	81.5[a]	62.3[b]	62.7[b]	57.7[b]	43.1[c]	3.84	0.002	<0.001	0.797

DM = dry matter; SEM = standard error of the mean.
[a–d]Values within a row with no common superscripts differ significantly ($P < 0.05$).

($P < 0.001$). In the present study, there were significant effects of TB on neither ratio of EAA, UAA and SAA to εAAs nor on ratio of EAA to NEAA.

Effects of supplementing TB on FAs composition in LTL muscle of weaned lambs

As shown in Table 7, the LTL muscle of lambs fed TB had higher content of saturated fatty acids (SFAs) such as C4:0 ($P < 0.001$), C10:0 ($P = 0.001$), C12:0 ($P < 0.001$), C13:0 ($P < 0.001$), C14:0 ($P = 0.001$), C15:0 ($P = 0.002$), C16:0 ($P = 0.003$), C17:0 ($P < 0.001$), C18:0 ($P < 0.001$), C20:0 ($P = 0.013$), C22:0 ($P = 0.002$) and C23:0 ($P < 0.001$). Despite the LTL muscle of lambs fed TB had lower content of UFAs ($P = 0.002$) including monounsaturated fatty acid (MUFA; $P = 0.045$) and polyunsaturated fatty acid (PUFA; $P < 0.001$) in comparison to that in the LTL muscle of lambs fed no TB, but the LTL muscle of lambs fed TB had higher content of conjugated linoleic acid (CLA; $P = 0.002$) such as t7,c9-CLA ($P = 0.041$), c9,t11-CLA ($P = 0.003$) and t11,c13-CLA ($P = 0.001$). Supplementing TB decreased ratios of both MUFA ($P < 0.001$) and PUFA ($P < 0.001$) to SFA. Besides, the PUFA contents of n3 ($P < 0.001$) and n6 ($P < 0.001$) as well as the ratio of n6 to n3 ($P < 0.001$) were linearly decreased with increasing TB supplementation, while atherogenicity index (AI; $P < 0.001$) and thrombogenicity index (TI; $P < 0.001$) were linearly increased.

Effects of supplementing TB on gene expression and nucleotide content in LTL muscle

Gene expression in Table 8 showed that supplementing TB upregulated the relative expressions of *SREBP-1C* ($P < 0.001$), *SCD* ($P = 0.019$), *PPARγ* ($P = 0.020$), *FAS* ($P < 0.001$) and *LPL* ($P < 0.001$) in LTL muscle of weaned lambs. As shown in Fig. 1, the LTL muscle of lambs fed with 4.0 g/kg TB had higher content of 5′-IMP ($P < 0.001$) but lower 5′-GMP ($P = 0.015$) compared with that of lambs without TB.

Discussion

Effects of supplementing TB on serum biochemical indices of weaned lambs

Gao et al. (2023) reported that dietary supplementation with 1.5 g/kg TB significantly increased IgM levels in the serum of weaned female calves. In the present study, lambs fed TB also were observed to have higher levels of IgM as well as IgA and IgG in serum, and this probably be related to the improvement of TB on gastrointestinal microflora and morphology. Our previous studies showed that TB could improve rumen microbial growth, which results in higher concentration of VFAs in the rumen (Ren et al. 2018b, 2018a). The VFAs including acetic acid, propionic acid, butyrate, valeric acid and branch-chained VFAs are typical organic acids, and they have been proved to increase the serum concentrations of IgM and IgG in weaned piglets (Long et al. 2017). Furthermore, Allaire et al. (2018) pointed out that there is a positive correlation between immunity and the development of intestinal epithelium, which acts as a physical barrier and a coordinating hub for immune defense and crosstalk between bacteria and immune cells. So far, TB has been demonstrated to improve the development and health of intestine by stimulating colonization of VFAs-producing bacteria, enhancing barrier functions of intestine and suppressing inflammatory responses in pre-weaned dairy

Table 6. The effects of tributyrin on amino acids (AAs) content in longissimus thoracis et lumborum muscle of Small-Tailed Han lambs

	Tributyrin additions, g/kg DM basis						*P*-values[1]		
Items	0	0.5	1.0	2.0	4.0	SEM	Contrast	Linear	Quadratic
ε EAAs (g/100 g LTL)	7.96[c]	8.41[bc]	8.73[abc]	9.25[a]	8.87[ab]	0.261	0.005	0.002	0.521
Valine	1.02	1.03	1.04	1.06	1.04	0.021	0.354	0.254	0.928
Methionine	0.42[c]	0.47[bc]	0.50[ab]	0.56[a]	0.52[ab]	0.019	<0.001	<0.001	0.429
Isoleucine	0.95[c]	0.99[bc]	1.00[abc]	1.04[a]	1.02[ab]	0.015	0.001	<0.001	0.168
Leucine	2.01[b]	2.08[ab]	2.02[b]	2.29[ab]	2.35[a]	0.106	0.140	0.010	0.248
Phenylalanine	0.96[b]	1.05[ab]	1.18[ab]	1.24[a]	1.09[ab]	0.075	0.034	0.050	0.953
Lysine	1.68[b]	1.84[ab]	2.06[ab]	2.17[a]	1.92[ab]	0.132	0.030	0.006	0.903
Threonine	0.90[ab]	0.91[a]	0.91[a]	0.88[b]	0.90[ab]	0.007	0.917	0.210	0.276
ε NEAAs (g/100 g LTL)	14.9[c]	15.8[b]	16.2[b]	16.6[a]	16.6[a]	0.147	<0.001	<0.001	0.334
Serine	0.73[b]	0.75[ab]	0.75[ab]	0.77[a]	0.74[ab]	0.012	0.100	0.232	0.017
Proline	0.82[b]	0.89[a]	0.88[a]	0.93[a]	0.92[a]	0.015	<0.001	<0.001	0.057
Glutamic acid	3.16[c]	3.21[bc]	3.24[bc]	3.38[a]	3.29[ab]	0.037	0.004	<0.001	0.120
Glycine	1.40[b]	1.61[a]	1.54[a]	1.56[a]	1.63[a]	0.041	<0.001	0.002	0.216
Histidine	0.67[c]	0.73[bc]	0.78[ab]	0.81[a]	0.80[a]	0.020	<0.001	<0.001	0.873
Alanine	0.77[c]	0.80[bc]	0.82[b]	0.83[b]	0.87[a]	0.011	<0.001	<0.001	0.407
Arginine	1.31[c]	1.39[bc]	1.44[ab]	1.50[a]	1.46[ab]	0.032	<0.001	<0.001	0.520
Aspartic acid	1.74[c]	1.83[bc]	1.92[b]	2.01[ab]	2.03[a]	0.033	<0.001	<0.001	0.856
Cystine	0.75[b]	0.81[ab]	0.86[a]	0.87[a]	0.90[a]	0.029	0.001	<0.001	0.802
Tyrosine	3.60[b]	37.8[ab]	3.93[a]	3.97[a]	3.97[a]	0.112	0.014	0.010	0.854
εAAs (g/100 g LTL)	22.9[d]	24.2[c]	24.9[bc]	25.9[a]	25.5[ab]	0.306	<0.001	<0.001	0.313
Branched-chain amino acids[2] (g/100 g LTL)	3.99[b]	4.11[ab]	4.06[b]	4.39[a]	4.42[a]	0.107	0.038	0.001	0.176
Umami amino acids[3] (UAAs, g/100 g LTL)	4.90[c]	5.05[b]	5.17[b]	5.40[a]	5.32[a]	0.051	<0.001	<0.001	0.213
Sweet amino acids[4] (SAAs, g/100 g LTL)	4.64[c]	4.99[ab]	4.92[b]	4.99[ab]	5.08[a]	0.036	<0.001	<0.001	0.031
EAA/*εAAs*, g/g	0.34	0.34	0.35	0.35	0.34	0.007	0.575	0.562	0.732
EAA/*NEAA*, g/g	0.53	0.53	0.54	0.55	0.53	0.016	0.643	0.582	0.720
UAA/*εAAs*, g/g	0.21	0.20	0.20	0.20	0.20	0.002	0.057	0.203	0.921
SAA/*εAAs*, g/g	0.203	0.206	0.197	0.192	0.199	0.003	0.216	0.037	0.789

DM = dry matter; SEM = standard error of the mean; EAAs = essential amino acids; NEAAs = nonessential amino acids.
[a-d]Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Linear, linear effect of tributyrin; Quadratic, quadratic effect of tributyrin.
[2]Branched-chain amino acids including valine, isoleucine and leucine.
[3]Umami amino acids including both glutamic acid and aspartic acid.
[4]Sweet amino acids including threonine, serine, glycine, alanine and proline.

calves (Liu et al. 2022). Therefore, supplementing TB was beneficial to enhance immune function of lambs, and this was consistent with the findings of Liu et al. (2021), who found that increasing TB addition in pasteurized waste milk could linearly enhance the health of dairy calves.

A previous study demonstrated that concentration of blood urea nitrogen can reflect effective utilization of dietary protein and ability of nitrogen retain in animal body, which is negatively correlated with feed efficiency (Coma et al. 1995; Whang and Easter 2000). Recently, our experiment demonstrated that supplementing TB not only enhanced feed efficiency by decreasing ratio of feed to body weight but also increased daily body weight again of weaned lamb (Li et al. 2023). This means that much of nitrogen from the diet were retained and used to synthesize muscle protein, which may result in a relatively lower concentration of blood urea nitrogen in lambs fed TB. In the present study, higher serum concentration of HDL in TB treatments was observed and this may be due to the stimulating function of TB on serum HDL (Sotira et al. 2020). A previous finding of Nazih et al. (2001) reported that butyrate can significantly increase the synthesis and secretion of ApoA-IV protein, which is the major component of HDL. Since supplementing TB could effectively promote the formation of butyrate in the rumen (Li et al. 2023); thus, TB is beneficial for promoting the concentration of ApoA-IV-containing HDL. AST and ALT are generally considered as indicators of potential liver damage. The present data showed that TB had no negative effects on hepatic functionality, and this in line with the reports of Sotira et al. (2020). It is worth mentioning that so far there have been limited

Table 7. The effects of tributyrin on fatty acid content in longissimus thoracis et lumborum muscle of Small-Tailed Han lambs

	Tributyrin additions, g/kg DM basis						*P*-values[1]		
Items	0	0.5	1.0	2.0	4.0	SEM	Contrast	Linear	Quadratic
εSFAs (mg/100 g LTL)	2357[c]	2406[c]	2604[b]	2643[b]	2755[a]	25.5	<0.001	<0.001	0.014
C4:0	5.3[c]	5.9[b]	6.0[b]	5.8[b]	6.4[a]	0.09	<0.001	<0.001	0.302
C10:0	7.6[c]	8.1[bc]	8.6[b]	8.1[bc]	9.5[a]	0.25	0.001	<0.001	0.067
C12:0	7.1[d]	8.6[bc]	8.5[c]	9.3[b]	10.2[a]	0.27	<0.001	<0.001	0.107
C13:0	14.1[c]	15.0[c]	19.7[b]	19.6[b]	21.9[a]	0.47	<0.001	<0.001	<0.001
C14:0	141[c]	144[c]	152[bc]	156[b]	180[a]	4.1	0.001	<0.001	0.321
C15:0	19.9[b]	20.1[b]	22.1[a]	22.9[a]	23.4[a]	0.59	0.002	<0.001	0.469
C16:0	1260[b]	1271[b]	1288[b]	1290[b]	1334[a]	10.6	0.003	<0.001	0.375
C17:0	55.3[b]	55.5[b]	60.6[b]	68.0[a]	71.1[a]	2.0	<0.001	<0.001	0.835
C18:0	765[b]	786[b]	941[a]	955[a]	987[a]	20.1	<0.001	<0.001	0.012
C20:0	2.8[b]	2.9[b]	3.2[b]	3.1[b]	3.7[a]	0.12	0.013	<0.001	0.143
C21:0	14.0[b]	15.0[ab]	14.9[ab]	16.5[ab]	17.4[a]	0.88	0.053	0.004	0.513
C22:0	5.3[c]	5.9[bc]	5.8[bc]	6.4[ab]	6.9[a]	0.26	0.002	<0.001	0.378
C23:0	59.3[c]	68.4[b]	72.2[b]	80.4[a]	83.3[a]	2.75	<0.001	<0.001	0.407
εUFAs (mg/100 g LTL)	2717[a]	2594[ab]	2511[ab]	2472[b]	2222[c]	74.1	0.002	<0.001	0.678
C14:1	13.8[a]	12.4[a]	11.3[a]	7.5[b]	8.7[b]	0.87	<0.001	<0.001	0.156
C15:1	12.9[a]	9.2[b]	7.7[b]	7.0[bc]	4.7[c]	0.86	<0.001	<0.001	0.848
C16:1n9c	115.4[a]	109.6[a]	90.5[b]	88.8[b]	68.8[c]	2.43	<0.001	<0.001	0.002
C17:1	43.3[a]	41.7[a]	37.0[b]	33.4[c]	31.5[c]	1.19	<0.001	<0.001	0.719
C20:1n9	10.0[a]	8.9[b]	7.2[c]	6.9[c]	5.3[d]	0.31	<0.001	<0.001	0.068
C18:1n9t	263[a]	252[a]	227[b]	208[c]	192[c]	6.2	<0.001	<0.001	0.118
C18:1n9c	1786[a]	1749[ab]	1746[ab]	1735[ab]	1596[b]	67.7	0.013	0.041	0.897
C18:2n6t	21.6[a]	21.5[a]	16.6[b]	14.7[b]	11.7[c]	0.79	<0.001	<0.001	0.071
C18:2n6c	323[a]	261[b]	243[bc]	235[bc]	190[c]	21.8	<0.001	<0.001	0.922
C18:3n3	13.9[a]	13.3[a]	10.2[b]	8.9[b]	6.7[c]	0.51	<0.001	<0.001	0.121
C18:3n6	7.2[a]	6.4[b]	7.0[ab]	6.3[b]	4.9[c]	0.26	<0.001	<0.001	0.146
C20:2	7.9[a]	6.7[ab]	5.4[bc]	4.8[cd]	3.9[d]	0.48	<0.001	<0.001	0.651
C20:3n6	10.5[a]	5.3[b]	5.0[b]	3.1[b]	1.5[b]	1.81	0.001	<0.001	0.546
C20:5n3	6.2[a]	5.6[ab]	4.4[bc]	3.6[c]	2.1[d]	0.48	<0.001	<0.001	0.585
C24:1	11.7[a]	11.8[a]	8.1[b]	6.9[b]	4.8[c]	0.54	<0.001	<0.001	0.044
C22:6 n3	8.5[a]	4.9[bc]	6.9[ab]	5.8[b]	3.0[c]	0.71	<0.001	<0.001	0.091
εCLA	71.3[c]	76.2[bc]	76.5[bc]	80.6[ab]	86.3[a]	2.20	0.001	<0.001	0.590
t7,c9-CLA	10.6[b]	10.7[b]	10.8[b]	10.8[b]	11.2[a]	0.08	0.041	<0.001	0.327
c9,t11-CLA	35.9[c]	39.5[bc]	39.1[bc]	42.9[ab]	46.6[a]	1.75	0.003	<0.001	0.392
t10,c12-CLA	12.8	13.7	13.8	13.8	13.4	0.70	0.265	0.606	0.856
t11,c13-CLA	11.9[c]	12.1[bc]	12.7[bc]	13.0[b]	15.1[a]	0.34	0.001	<0.001	0.305
MUFAs [2] (mg/100 g LTL)	2246[a]	2192[a]	2136[a]	2108[ab]	1912[b]	69.5	0.045	0.001	0.693
PUFAs [3] (mg/100 g LTL)	471[a]	401[b]	375[b]	363[bc]	310[c]	21.3	<0.001	<0.001	0.876
MUFA/SFA, mg/mg	0.95[a]	0.91[a]	0.82[b]	0.79[b]	0.69[c]	0.029	<0.001	<0.001	0.299
PUFA/SFA, mg/mg	0.19[a]	0.17[b]	0.14[bc]	0.13[cd]	0.11[d]	0.008	<0.001	<0.001	0.594
ε n3 (mg/100 g LTL)	28.6[a]	23.8[ab]	21.5[bc]	18.2[bc]	11.7[c]	2.27	<0.001	<0.001	0.766

(*Continued*)

Table 7. (*Continued.*)

Items	Tributyrin additions, g/kg DM basis					SEM	*P*-values[1]		
	0	0.5	1.0	2.0	4.0		Contrast	Linear	Quadratic
ε n6 (mg/100 g LTL)	363[a]	294[b]	271[b]	259[bc]	208[c]	20.9	<0.001	<0.001	0.920
ε n6/ε n3, mg/mg	12.6[b]	12.9[b]	12.6[b]	14.2[b]	18.0[a]	1.08	0.135	<0.001	0.844
Atherogenicity index[4], mg/mg	0.71[c]	0.75[bc]	0.78[b]	0.81[b]	0.96[a]	0.024	<0.001	<0.001	0.383
Thrombogenicity index[5], mg/mg	1.49[c]	1.58[c]	1.77[b]	1.83[b]	2.12[a]	0.052	<0.001	<0.001	0.184

CLA = conjugated linoleic acid; DM = dry matter; SEM = standard error of the mean; SFAs = saturated fatty acids; UFAs = unsaturated fatty acids.
[a-d]Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1]Linear, linear effect of tributyrin; Quadratic, quadratic effect of tributyrin.
[2]Monounsaturated fatty acids including C14:1, C15:1, C16:1n9c, C17:1, C20:1, C18:1n9t, C18:1n9c and C24:1.
[3]Polyunsaturated fatty acids including C18:2n6t, C18:2n6c, C18:3n6, C20:2, C18:3n3, C18:3n6, C20:5n3, C22:6n3 and CLA.
[4]Atherogenicity index = (C12:0 + 4 × C14:0 + C16:0)/ (MUFA + PUFA) calculated according to Ulbricht and Southgate (1991).
[5]Thrombogenicity index = (C12:0 + C16:0 + C18:0)/ [(0.5 × MUFA) + (0.5 × *n* − 6 PUFA) + (3 × *n* − 3 PUFA) + (*n* − 3 PUFA/*n* − 6 PUFA)] calculated according to Ulbricht and Southgate (1991).

Table 8. The effects of tributyrin on the relative expressions of sterol regulatory element binding protein 1C (SREBP-1C), stearoyl-CoA desaturase (SCD), peroxisome proliferator-activated receptor γ (PPARγ), acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS) and lipoprotein lipase (LPL) in longissimus thoracis et lumborum of Small-Tailed Han lambs

Items	Tributyrin additions, g/kg DM basis					SEM	*P*-values		
	0	0.5	1.0	2.0	4.0		Contrast	Linear	Quadratic
SREBP − 1C	0.91[c]	0.94[bc]	1.48[a]	1.03[b]	1.01[bc]	0.035	<0.001	0.019	<0.001
SCD	0.64[b]	0.86[ab]	1.03[a]	0.93[ab]	1.05[a]	0.107	0.015	0.019	0.446
PPARγ	1.19[b]	1.34[b]	2.15[a]	1.29[b]	1.48[b]	0.128	0.020	0.222	<0.001
ACC	0.88	0.81	1.01	0.86	0.93	0.082	0.862	0.587	0.084
FAS	0.71[c]	0.78[b]	0.83[ab]	0.80[b]	0.90[a]	0.024	<0.001	<0.001	0.191
LPL	1.03[c]	1.23[b]	1.44[a]	1.08[c]	1.26[b]	0.038	<0.001	0.024	<0.001

DM = dry matter; SEM = standard error of the mean.
[a–c]Values within a row with no common superscripts differ significantly ($P < 0.05$).

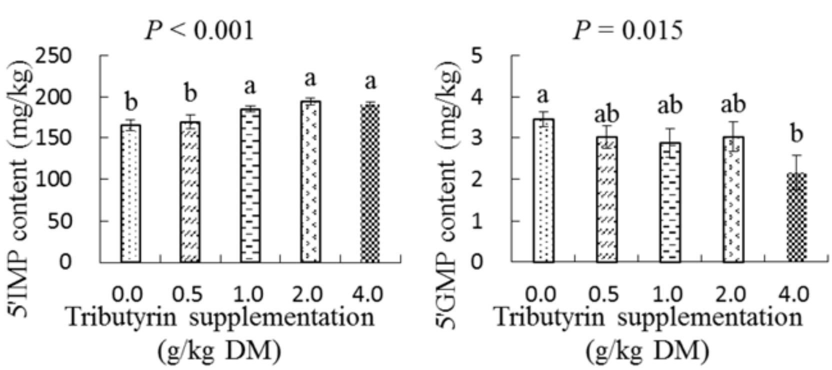

Figure 1. The effects of tributyrin on contents of inosine-5′-phosphate (5′-IMP) and guanosine-5′-monophosphate (5′-GMP) of longissimus thoracis et lumborum of Small-Tailed Han lambs.
[a-b]Values within the tributyrin treatments with no common superscripts differ significantly ($P < 0.05$).

studies investigating the effects of supplementing TB on serum biochemical indices such as serum immunoglobulin, urea and HDL in ruminant animals, and more research is required to determine the potential mechanism of how TB influence the serum indices.

Effects of supplementing TB on fat accumulation and gene expression in LTL muscle of weaned lambs

In the present experiment, TB increased content of ether extract and intermuscular fat length in LTL muscle, and this may be associated with the elevating expressions of *LPL, PPARγ, SREBP-1C, SCD* and *FAS*. So far, little is known about the benefit of TB added into solid feed on regulation of the genes related to lipid metabolism, but some useful information can be obtained from the regulation and mechanism of butyrate on fat metabolism. Xu et al. (2022) reviewed that butyrate could stimulate fat accumulation by activating G-protein coupled receptors. Once the receptors are activated, they will regulate the downstream pathway by inhibiting the activity of adenylate-activated protein kinase which is involved in the regulation of fat metabolism, thereby inducing lipid accumulation in cells. The aforementioned regulating role of butyrate was proved by Cheng et al. (2020), who reported that 1.0 mmol/L butyrate could promote de novo synthesis of milk fat in bovine mammary epithelial cells by inhibiting the activity of adenylate-activated protein kinase and upregulating the expression of *SREBP-1C*. In addition, Kong (2012) demonstrated that 0.75 mmol/L butyrate could effectively upregulate the mRNA expressions of *FAS* and *acetyl-CoA carboxylase*, thus resulting in an increasing milk fat biosynthesis in bovine mammary epithelial cells. Our previous experiment demonstrated that supplementing TB significantly promoted butyrate concentration in the rumen of Small-Tailed Han sheep (Ren et al. 2018b), which is beneficial to upregulate the mRNA expression of the present target genes such as *FAS* and *SREBP-1C* in LTL muscle of lambs.

Impacts of TB on meat quality characteristics of LTL muscle

In the current study, supplementing TB increased both calcium and phosphorus levels in LTL muscle, and this may be associated with the improvement of TB on gastrointestinal development of lambs. It well known that absorption of calcium and phosphorus occurs primarily in the small intestine of ruminants, with small amounts absorbed in the rumen (Veum 2010). Liu et al. (2022) reported that TB could effectively improve the development of small intestine of pre-weaned dairy calves. Recently, supplementing TB had been demonstrated that it not only stimulated intestinal development but also accelerated rumen development of weaned lambs (Li et al. 2023), which was beneficial for absorption of dietary calcium and phosphorus. Interestingly, our previous study also indirectly proved that supplementing TB could effectively enhance the daily retentions of dietary calcium and phosphorus in ewes by reducing excretions of calcium and phosphorus in feces and urine (Ren et al. 2018b), which was beneficial to accumulate higher calcium and phosphorus in LTL muscle.

In the present study, the LTL muscle of lambs fed diets with supplementation of TB had higher pH values, and this may be due to the inhibiting effect of TB on lactate dehydrogenase activity. The lactate dehydrogenase is a tetrameric enzyme, which converts pyruvate to lactate (Le et al. 2010). As muscle is converted to meat, a shift occurs from aerobic to anaerobic metabolism, which favors the production of lactic acid, resulting in the declining pH of the tissue (Huff-Lonergan and Lonergan 2005). The present serum biochemical indices showed that lambs fed TB had lower lactate dehydrogenase activity, which was beneficial to reduce the decline of pH in LTL muscle. Meat quality characteristics including meat color, water holding capacity and shear force are affected by many factors such as postmortem aging and anti-oxidative stability of muscle (Dou et al. 2022). In the present study, supplementing TB was observed to markedly elevate muscle redness of lambs, and this may be associated with the anti-oxidative function of TB. Gao et al. (2023) demonstrated that supplementing TB could significantly reduce the levels of both reactive oxygen species and malonaldehyde while increase superoxide dismutase in the blood of weaned calves. Thus, supplementing TB was beneficial to improve meat redness of lambs. The current results were consistent with the findings of Wang et al. (2024), who reported that supplementing TB with dosages ranged from 0.5 to 4.0 g/kg could significantly increase the pH, redness and water holding capacity of foreshank muscle of weaned Small-Tailed Han sheep lambs. Interestingly, the present study showed that TB also affected the eating quality of LTL muscle of lambs via decreasing hardness, cohesiveness, springiness, gumminess and chewiness, and this may be due to the enhancing effects of TB on water holding capacity in LTL muscle. It is well known that a higher water content in cooked meat can result in greater tenderness, while the meat with a lower water holding capacity may result in large reductions in water content and which is expected to increase its hardness, cohesiveness, springiness, gumminess and chewiness (Yu et al. 2021). Based on the present results, TB as an effective feed additive had good potential to improve both nutritional and eating quality of LTL muscle of weaned lambs.

Effects of TB on AAs and nucleotides content in LTL muscle

In the present test, supplementing TB was observed to effectively increase contents of total AAs particularly EAAs in LTL muscle, and this may be associated with positive effect of supplementing TB on ruminal MCP synthesis. It is well known that the MCP synthesis plays a key nutritional role in meeting AAs required by ruminants, which evenly could provide 81% of the qualitative AA requirements of growing lambs (Nolte 2006). Furthermore, the synthesized MCP in the rumen is also an excellent protein source since it has a relatively good AA balance and digestibility compared with cereal protein (Firkins 1996). Sok et al. (2017) pointed out that the synthesized MCP in the rumen contains at least 18 type AAs including 7 EAAs detected in the current experiment. Our previous experiment showed that ewes fed TB had higher daily yield of MCP in the rumen (Ren et al. 2018a); thus, supplementing TB was beneficial to the biosynthesis of AAs in LTL muscle by contributing more MCP yield for lambs. The present results agreed with the reports of Wang et al. (2024), who reported that supplementing TB could enhance the biosynthesis of AAs in foreshank muscle of weaned lambs.

Nakatani et al. (1986) reported that 5′-IMP is responsible for the umami taste of meat, and the more 5′-IMP the meat contain the better meat taste. But the meat content of 5′-IMP is varied with individual sample, which is usually affected by many factors such as animal breed, age, sex, feed, tissue position, cooking conditions and so on (Zhang et al. 2021). The present study showed that supplementing TB with dosages ranged from 1.0 to 4.0 g/kg could increase the 5′-IMP level in the LTL muscle, and this may because of the antioxidative effects of TB. With bio-reactions of metabolic enzymes in muscle, ATP may be degraded to ADP after slaughter, following generations of AMP and IMP (Nakatani et al. 1986). The generated IMP can be further catalyzed into xanthosine monophosphate by IMP dehydrogenase to produce 5′-GMP (Li et al. 2018). But the IMP dehydrogenase can be well accumulated in response to oxidative or replicative stress (Van der Knaap and Verrijzer 2016). Since TB has been shown to effectively enhance the antioxidant status by reducing the level of reactive oxygen species while increasing superoxide dismutase in serum of calves (Gao et al. 2023); thus, dietary supplementation with TB may reduce the accumulation of the IMP dehydrogenase, which could result in more contents of IMP and lower contents of 5′-GMP generated in muscle. The present study indicated that TB could improve umami taste of mutton by increasing accumulation of 5′-IMP in LTL muscle.

Effects of TB on FAs composition in LTL muscle

Recently, more and more evidences showed that FA profiling such as SFA, MUFA and PUFA in ruminant meat could be modified by ruminal microbiome. For example, *Christensenellaceae_R-7_group* derived from the rumen of Hu sheep had been demonstrated to be positively correlated with the level of n3-PUFA in foreshank muscle (Xiong et al. 2021), while *Quinella, Ruminococcus 2* and *coprostanoligenes* (*Eubacterium*) were showed to be positively correlated with the content of linoleic acid in longissimus lumborum of Black Tibetan sheep by Zhang et al. (2022). Currently, supplementing TB was observed to modify the FA composition in LTL muscle of weaned lambs by increasing the content of SFAs while decreasing UFAs, and this may be due to the effects of TB on the changing relative abundances of rumen bacteria responsible for FA biohydrogenation. Potu et al. (2011) pointed out that rumen bacteria especially fibrolytic bacteria such as *Fibrobacter, Ruminococcus* and *Butyrivibrio* are important in the biohydrogenation process of dietary UFA. For example, C15:1 can be converted to C15:0 by *Fibrobacter* (Zhang et al. 2017), while linoleic acid (C18:2n6c) can be bio-hydrogenated to produce C18:0 by *Butyrivibrio* (Wallace et al. 2006). Boeckaert et al. (2008) reported that *Butyrivibrio* is also the principal rumen bacteria involved in biohydrogenation of C18:1. In addition, a study of Jeyanathan et al. (2016) demonstrated that C22:6$n-3$ is also bio-hydrogenated by *Butyrivibrio* to produce 22 carbon FAs such as C22:0. Recently, Li et al. (2023) reported that the relative abundances of *Butyrivibrio, Streptococcus* and *Fibrobacter* could be enhanced by supplementing TB in diet of lambs, which may accelerate the microbial biohydrogenation of UFAs and formation of SFAs in the rumen. Despite SFAs such as C18:0, C16:0 and C14:0 are commonly considered as harmful FAs to human health, but the SFAs could provide more energy value, have higher resistance to reduce oxidation and own greater octane number for better combustion efficiency (Liu et al. 2019).

Conjugated linoleic acid has high health amelioration potentials; hence, there is of great interest to increase the CLA content in meat. In the current experiment, TB could increase the content of CLA in LTL, and this may be associated with the stimulating effects of TB on biohydrogenation of the rumen bacteria. It well known that the CLA is one of the intermedia biohydrogenated in the rumen, and its level in the meat is related to the microbial isomerization of C18:2n6 in the rumen (Bessa et al. 2000). In addition, TB has been proved to stimulate both rumen and intestine developments via stimulating VFA-producing bacteria (Li et al. 2023), and this was also beneficial for the absorption and accumulation of the CLA isomers in LTL muscle. AI and TI are related to the profile of FAs, which could be decreased by the high content of UFA particularly PUFA (Ulbricht and Southgate 1991). Since TB could effectively decrease the content of UFAs, lambs fed TB had both higher AI and TI.

Conclusions

This study showed that supplementing TB could affect the serum biochemical indices of weaned lambs by enhancing serum concentrations of immunoglobulins, minerals and HDL while decreasing urea and lactate dehydrogenase activity. Besides, TB additions may improve pH value, redness, water holding capacity and intermuscular fat length in LTL muscle, but TB reduced the muscle shear force and texture. In addition, TB increased the content of 5′-IMP in the muscle. The mostly important, TB could increase EAAs content of the LTL muscle. Furthermore, TB could change the muscle FAs composition by increasing SFAs' level as well as CLA content. The determined genes related to FAs metabolism showed that supplementing TB could upregulate the relative expressions of *SREBP-1C, SCD, PPARγ, FAS* and *LPL*. Above results indicated that supplementing TB not only could promote the healthy status of weaned lambs via promoting serum immunity but also can improve the nutritional quality of the LTL muscle by improving EAAs content as well as CLA level.

Author contributions. **Qing-Chang Ren**: Conceptualization, funding acquisition, reviewing and editing; **Ya-Xin Wang** and **Xue-Er Wang**: Original writing, Determinations of amino acid, fatty acid and genes; **Zhi-Wei Li and Ran An**: Determination of chemicals and serum biochemical indices; **Jian-Zhuang Tan**: Providing important contributions during measurements of both amino acid and fatty acid.

Ya-Xin Wang and Xue-Er Wang authors are contributed equally to this study.

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