

Effects of Different Feed Additives on Fatty Acid Composition and Oxidative Stability of Body Fat in Duhan Hybrid Mutton Sheep: Postprint

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Abstract

This experiment aimed to investigate the effects of different feed additives on the fatty acid composition and oxidative stability of body fat in Dorper × Han hybrid mutton sheep. Thirty healthy 6-month-old Dorper × Han F1 hybrid mutton sheep with similar body weight [(42.5±\$3.1) kg] were selected and divided into 3 groups with 10 sheep per group using a single-factor completely randomized block design. The control group (G1) was fed a basal diet, experimental group 1 (G2) was fed the basal diet supplemented with *Allium mongolicum* powder at 20 g per sheep per day, and experimental group 2 (G3) was fed the basal diet supplemented with microbial fermented feed at 100 g per sheep per day. The experimental period lasted 75 days, including a 15-day preliminary period and a 60-day formal experimental period. After the feeding trial, 3 sheep were randomly selected from each group for slaughter, and fat samples were collected from perirenal, subcutaneous abdominal, and tail regions to determine fatty acid composition, malondialdehyde (MDA) content, and superoxide dismutase (SOD) activity. The results showed that compared with G1: 1) G2 and G3 had significantly higher average daily gain ($P<0.05$) and significantly lower feed conversion ratio ($P<0.05$); 2) G2 exhibited significantly higher contents of linoleic acid (C18:2cis-6), α -linolenic acid (C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosahexaenoic acid (DHA, C22:6n-3), polyunsaturated fatty acids (PUFA), n-6 PUFA, and PUFA/saturated fatty acids (SFA) ratio ($P<0.05$), and significantly lower stearic acid (C18:0) content ($P<0.05$) in perirenal, subcutaneous abdominal, and tail adipose tissues, while G3 showed significantly higher DHA content in subcutaneous abdominal and tail adipose tissues ($P<0.05$) and significantly lower monounsaturated fatty acids (MUFA) and C18:0 contents in perirenal adipose tissue ($P<0.05$); 3) G2 and G3 had significantly higher n-6 PUFA content and n-6/n-3 ratio in tail adipose tissue ($P<0.05$), G2 had significantly higher n-3 PUFA content in

perirenal adipose tissue ($P<0.05$), and G3 had significantly lower PUFA/SFA ratio in perirenal adipose tissue ($P<0.05$); 4) G2 and G3 showed significantly higher SOD activity and significantly lower MDA content in subcutaneous abdominal and perirenal adipose tissues ($P<0.05$); 5) Linear relationships existed between MDA content, SOD activity, and PUFA deposition in subcutaneous abdominal ($R^2=0.967$), tail ($R^2=0.965$), and perirenal adipose tissues ($R^2=0.992$) ($P<0.001$), with MDA contributing negatively and SOD contributing positively to the multiple regression equations. In conclusion, dietary supplementation with *Allium mongolicum* powder and microbial fermented feed can improve growth performance in intensively reared Dorper \times Han hybrid mutton sheep; dietary *Allium mongolicum* powder supplementation can effectively improve body fatty acid composition and enhance fat oxidative stability, thereby improving fat quality; dietary microbial fermented feed supplementation has limited effects on improving body fatty acid composition but can enhance fat oxidative stability in Dorper \times Han hybrid mutton sheep.

Full Text

Effects of Different Feed Additives on Fatty Acid Composition and Oxidation Stability of Body Fat in Dorper \times Thin-Tailed Han Crossbred Mutton Lambs

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Abstract

This experiment aimed to investigate the effects of different feed additives on fatty acid composition and oxidation stability of body fat in Dorper \times thin-tailed Han crossbred mutton lambs. Thirty healthy, six-month-old Dorper \times thin-tailed Han crossbred mutton lambs with similar body weight [(42.5 \pm \$3.1) kg] were randomly allocated to three groups (n=10) using a single-factor completely randomized block design. Lambs in the control group (G1) were fed a basal diet, those in experimental group 1 (G2) received the basal diet supplemented with *Allium mongolicum* Regel powder at 20 g per lamb per day, and those in experimental group 2 (G3) received the basal diet supplemented with microbial fermented feed at 100 g per lamb per day. The trial lasted 75 days, including a 15-day pre-feeding period and a 60-day formal experimental period. At the end of the feeding trial, three lambs were randomly selected from each group for slaughter. Fat samples were collected from the perirenal, abdominal subcutaneous, and tail regions to determine fatty acid composition, malondialdehyde (MDA) content, and superoxide dismutase (SOD) activity.

Compared with G1, the results showed: 1) G2 and G3 exhibited significantly higher average daily gain and significantly lower feed-to-gain ratio ($P<0.05$). 2)

G2 showed significantly higher contents of linoleic acid (C18:2cis-6), α -linolenic acid (C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3), docosahexaenoic acid (DHA, C22:6n-3), polyunsaturated fatty acids (PUFA), n-6 PUFA, and PUFA/saturated fatty acid (SFA) ratio ($P < 0.05$), along with significantly lower stearic acid (C18:0) content in all three fat depots ($P < 0.05$). G3 demonstrated significantly higher DHA content in abdominal subcutaneous and tail fat ($P < 0.05$) and significantly lower MUFA and C18:0 content in perirenal fat ($P < 0.05$). 3) G2 and G3 showed significantly increased n-6 PUFA content and n-6/n-3 ratio in tail fat ($P < 0.05$). G2 exhibited significantly elevated n-3 PUFA content in perirenal fat ($P < 0.05$), while G3 showed significantly decreased PUFA/SFA ratio in perirenal fat ($P < 0.05$). 4) G2 and G3 significantly upregulated SOD activity and downregulated MDA content in abdominal subcutaneous and perirenal fat ($P < 0.05$). 5) Linear relationships existed between MDA content, SOD activity, and PUFA deposition in abdominal subcutaneous ($R^2 = 0.967$), tail ($R^2 = 0.965$), and perirenal fat ($R^2 = 0.992$) ($P < 0.001$), with MDA contributing negatively and SOD contributing positively to the regression equations.

In conclusion, dietary supplementation with both *Allium mongolicum* Regel powder and microbial fermented feed can improve growth performance in housed Dorper \times thin-tailed Han crossbred mutton lambs. *Allium mongolicum* Regel powder effectively improves fatty acid composition and oxidative stability of body fat, thereby enhancing fat quality. Microbial fermented feed shows limited effects on improving fatty acid composition but can enhance fat oxidative stability.

Keywords: feed additives; mutton lamb; fat quality; antioxidant; oxidation stability; polyunsaturated fatty acids

Introduction

The behavioral habits and foraging patterns of grazing sheep are far more complex and enriched than those under housed conditions, resulting in marked differences in meat quality between these two production systems, particularly in fatty acid composition and antioxidant content [1]. Given current national circumstances in China, grazing systems for mutton sheep are gradually being restricted [2], while intensive housed or semi-housed fattening has become the mainstream model in the sheep industry. However, excessive use of concentrate feed coupled with insufficient exercise in this model leads to excessive fat deposition, deteriorated mutton quality, low feed efficiency, and compromised production profitability. The fatty acid composition of livestock body fat forms the basis for the unique flavor of meat. Mutton flavor is primarily associated with short-chain branched fatty acids, stearic acid (C18:0), and oxidation degradation products of unsaturated fatty acids (UFA) in body fat [3-4]. Numerous studies have demonstrated that fat oxidation is a primary cause of quality de-

terioration during meat storage, with the balance between pro-oxidative and antioxidant components being most critical [5-6]. Polyunsaturated fatty acids (PUFA) serve as preferred substrates for initiating and accelerating lipid oxidation in muscle cell membranes, acting as oxidation enhancers alongside metal ions, heme proteins, and reactive oxygen species. As highly unsaturated components in meat products, PUFA are particularly vulnerable to oxidative attack, especially those in the polar fractions of phospholipids, which have the highest proportion and strongest oxidative sensitivity [7-8]. Oxidation severely compromises meat flavor and nutritional value, primarily unsaturated fatty acids and fat-soluble vitamins [9-10]. The degree of fat oxidation during storage directly determines sensory quality, accelerating color fading and producing off-flavors that reduce consumer acceptability [11]. Dietary nutrient composition plays a key role in fat metabolism, deposition, and antioxidant capacity [12], making nutritional regulation an effective approach to address declining quality in housed mutton production.

Both natural plant extracts and microbial fermented feed can improve meat fatty acid composition and oxidative stability to varying degrees, thereby extending shelf life. *Allium mongolicum* Regel, also known as Mongolian chive, is a natural high-quality forage that grows in arid regions such as deserts and wastelands [13]. *Allium mongolicum* and its extracts exhibit strong antioxidant activity and positively affect livestock product quality. They can significantly increase catalase (CAT) and total superoxide dismutase (T-SOD) activities in serum while reducing malondialdehyde (MDA) content in liver tissue, thereby enhancing antioxidant capacity in both sheep [14] and mice [15]. *Allium mongolicum* and its extracts also improve mutton fat quality [16], increasing PUFA and conjugated linoleic acid (CLA) contents while decreasing C18:0 content in intramuscular fat of mutton lambs [17]. They reduce saturated fatty acid (SFA) content while increasing monounsaturated fatty acid (MUFA) and PUFA contents in the longissimus dorsi muscle of housed mutton lambs, thus improving meat quality [18].

Microbial fermented feed is a non-toxic, residue-free green feed additive that improves dietary nutritional value and consequently affects meat fatty acid composition. Wang [19] found that fermented feed supplementation in Small-tailed Han sheep significantly increased oleic acid (C18:1cis-9) and α -linolenic acid (C18:3n-3) contents in muscle and kidney fat, improving mutton fatty acid composition. Lin et al. [20] reported that fermented feed in Duroc \times Landrace \times Yorkshire crossbred boars significantly increased C18:3n-3 and MUFA contents in muscle fat, enhancing nutritional value while reducing thiobarbituric acid reactive substances and slowing fat oxidation rate, thereby extending pork shelf life. Building on previous research, this study used Dorper \times thin-tailed Han crossbred lambs as an animal model to investigate the effects of *Allium mongolicum* Regel powder and microbial fermented feed on fatty acid composition and oxidative stability in different fat depots, and to reveal the relationship between PUFA deposition and oxidative performance indicators (MDA content and SOD activity), providing a theoretical basis for

further application of novel feed additives in housed mutton sheep production.

1.1 Experimental Period and Location

The experiment was conducted from July to October 2017 at the “China-Canada Mutton Sheep Breeding and Demonstration Project” base of Inner Mongolia Agricultural University.

1.2 Experimental Animals and Grouping

Thirty healthy, six-month-old Dorper×thin-tailed Han crossbred mutton lambs with similar body weight [(42.5±\$3.1) kg] were randomly allocated to three groups (n=10) using a single-factor completely randomized block design. The basal diet was a pelleted total mixed ration (TMR) for fattening sheep, manufactured by Inner Mongolia Youmote Animal Husbandry Technology Co., Ltd. The control group (G1) received the basal diet only. Experimental group 1 (G2) received the basal diet supplemented with *Allium mongolicum* Regel powder at 20 g per lamb per day (based on reference [21]). Experimental group 2 (G3) received the basal diet supplemented with microbial fermented feed at 100 g per lamb per day (manufacturer’s recommended dosage). *Allium mongolicum* Regel powder was purchased from Alxa League Haohai Biotechnology Co., Ltd., Inner Mongolia. Microbial fermented feed was produced by Zhumadian Jixinglong Animal Husbandry Biotechnology Co., Ltd., containing viable microorganisms (*Bacillus subtilis*, photosynthetic bacteria, yeast, *Bifidobacterium*, *Aspergillus niger*, etc.) and fermentation products (amino acids, organic acids, bacterial protein, cellobiose, bifidogenic factors, and immune/growth-promoting factors). The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (DM basis)

Items	Content
Ingredients	
Leymus chinensis	
Alfalfa	
Cracked corn	
Wheat bran	
Sunflower seed meal	
Pea stalk	
CaHPO ₄	
NaCl	
Premix ¹⁾	
Total	
Nutrient levels²⁾	
DE/(MJ/kg)	

Items	Content
CP	
EE	
NDF	
ADF	

¹⁾ The premix provided the following per kg of diet: Zn (as zinc sulfate) 30 mg, Fe (as ferrous sulfate) 26 mg, Cu (as copper sulfate) 7.9 mg, Mn (as manganese sulfate) 30 mg, I (as potassium iodide) 5.4 mg, Co (as cobalt sulfate) 0.1 mg, VA 3,500 IU, VE 25 IU, VD₃ 1,100 IU.

²⁾ DE was an estimated value, while the others were measured values.

1.3 Animal Management

Prior to the experiment, all lambs were dewormed and housing facilities were disinfected. The entire trial lasted 75 days, including a 15-day pre-feeding period and a 60-day formal experimental period. During the formal period, lambs were fed twice daily at 07:00 and 18:00, with 1.5 hours allowed for feeding. Residual feed was collected at 08:30 and 19:30 daily. Ad libitum access to feed and water was provided throughout the trial. All groups were managed under identical housing, feeding, and environmental conditions.

1.4 Sample Collection

At the end of the feeding trial, three lambs with similar body weight were randomly selected from each group and slaughtered after 24-hour feed withdrawal and 2-hour water withdrawal. Fat samples were collected from abdominal subcutaneous, tail central, and perirenal regions and stored at -20 °C.

1.5.1 Growth Performance Measurements

During the formal experimental period, residual feed was weighed and recorded daily before feeding. Average daily feed intake was calculated based on actual daily intake per group. Lambs were weighed every 15 days to record body weight changes.

1.5.2 Fatty Acid Composition Analysis

Approximately 60-70 mg of fat sample was weighed and mixed with 2 mL sodium hydroxide-methanol solution and 100 µL internal standard. The mixture was incubated in an 85 °C water bath for 30 minutes, followed by addition of 3 mL boron trifluoride-methanol solution and another 30-minute incubation at 85 °C. After cooling to room temperature, 1 mL n-hexane was added for extraction. After settling, 100 µL of the supernatant was collected and diluted to 1 mL with n-hexane for gas chromatography analysis. Fatty acid methyl esters were

analyzed using an Agilent GC 890N gas chromatograph with a TG-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The oven temperature program started at 80 °C for 1 minute, increased to 200 °C at 10 °C/min, then to 250 °C at 5 °C/min, and finally to 270 °C at 2 °C/min with a 3-minute hold. Helium was used as carrier gas at 1.2 mL/min. The injector temperature was 290 °C with splitless injection. Ion source and transfer line temperatures were both 280 °C, with an injection volume of 1 μL.

1.5.3 MDA Content and SOD Activity Assays

MDA content and SOD activity in fat samples from three depots were determined using commercial assay kits (MDA: A003-1; SOD: A001-1) from Nanjing Jiancheng Bioengineering Institute, following the manufacturer's instructions. Protein content in adipose tissue was measured using the Coomassie brilliant blue reagent kit from the same institute at 596 nm.

1.6 Statistical Analysis

Data were processed using Excel 2007. One-way ANOVA was performed using the ANOVA procedure in SAS 9.2 software. Differences were considered significant at $P < 0.05$, with Duncan's multiple comparison test applied when significant differences were detected. Multiple regression analysis was used to evaluate relationships between MDA content, SOD activity, and PUFA deposition in various fat depots.

2.1 Effects of Different Feed Additives on Growth Performance

As shown in Table 2, no significant differences were observed in average daily feed intake among groups ($P > 0.05$). Average daily gain in G2 and G3 was significantly higher than in G1 ($P < 0.05$), with no significant difference between G2 and G3 ($P > 0.05$). Feed-to-gain ratio in G2 and G3 was significantly lower than in G1 ($P < 0.05$), with no significant difference between G2 and G3 ($P > 0.05$).

Table 2 Effects of different feed additives on growth performance of Dorper × thin-tailed Han crossbred mutton lambs

Items	G1	G2	G3	P-value
Initial body weight (kg)	42.58 ^a ± 2.78	42.63 ± 3.57	42.95 ± 3.40	<i>Averagedailyfeedintake(kg/d)</i> 1.32 ± 0.19 1.33 ± 0.19
	<i>to – gainratio</i>	6.93 ± 0.99 ^a	5.70 ± 1.01 ^b	5.56 ± 1.28

Values in the same row with different superscripts differ significantly ($P < 0.05$). The same applies below.

2.2 Effects on Abdominal Subcutaneous Adipose Tissue Fatty Acid Composition, MDA Content, and SOD Activity

As shown in Table 3, 21 fatty acids were detected in abdominal subcutaneous adipose tissue. The most abundant SFA was palmitic acid (C16:0), followed by C18:0, while the predominant MUFA was C18:1cis-9. The major PUFA were linoleic acid (C18:2cis-6), arachidonic acid (C20:4n-6), C18:3n-3, and docosahexaenoic acid (DHA, C22:6n-3).

G2 showed significantly lower C18:0 content compared to G1 and G3 ($P < 0.05$), while G2 exhibited significantly higher contents of C18:2cis-6, C18:3n-3, eicosapentaenoic acid (EPA, C20:5n-3), and DHA ($P < 0.05$). No significant differences were observed between G1 and G3 for these fatty acids ($P > 0.05$). G2 had significantly lower SFA content and significantly higher PUFA, n-6 PUFA contents, and PUFA/SFA ratio compared to G1 and G3 ($P < 0.05$), with no significant differences between G1 and G3 ($P > 0.05$). G3 showed significantly higher MUFA content than G1 and G2 ($P < 0.05$).

MDA content in G2 was significantly lower than in G1 and G3 ($P < 0.05$), while G3 had significantly lower MDA content than G1 ($P < 0.05$). SOD activity in G2 was significantly higher than in G1 and G3 ($P < 0.05$), and G3 showed significantly higher SOD activity than G1 ($P < 0.05$).

Table 3 Effects of different feed additives on fatty acid composition, MDA content, and SOD activity in abdominal subcutaneous adipose tissue of Dorper × thin-tailed Han crossbred mutton lambs

[Table content preserved with English headers]

2.3 Effects on Tail Adipose Tissue Fatty Acid Composition, MDA Content, and SOD Activity

As shown in Table 4, 21 fatty acids were detected in tail adipose tissue. The most abundant SFA was C16:0, followed by C18:0, while C18:1cis-9 was the predominant MUFA. The major PUFA were C18:2cis-6, C20:4n-6, C18:3n-3, and γ -linolenic acid (C18:3n-6).

G2 showed significantly lower C18:0 content than G1 and G3 ($P < 0.05$). G3 had lower C18:0 than G1, but the difference was not significant ($P > 0.05$). G2 exhibited significantly higher contents of C18:2cis-6, C18:3n-3, C18:3n-6, EPA, and DHA compared to G1 and G3 ($P < 0.05$). Except for DHA, which differed significantly between G1 and G3 ($P < 0.05$), no significant differences were observed for other PUFA between these groups ($P > 0.05$). G2 had significantly lower SFA content than G1 and G3 ($P < 0.05$), with no significant difference between G1 and G3 ($P > 0.05$). G2 showed significantly higher MUFA, PUFA, n-6 PUFA, n-3 PUFA contents, and PUFA/SFA ratio than G1 and G3 ($P < 0.05$). In G3, only n-6 PUFA content and n-6/n-3 ratio were significantly higher than in G1 ($P < 0.05$), with no significant differences for other indices ($P > 0.05$).

MDA content in G2 was significantly lower than in G1 and G3 ($P < 0.05$), while G3 had significantly lower MDA content than G1 ($P < 0.05$). SOD activity in G2 was significantly higher than in G1 and G3 ($P < 0.05$), with no significant difference between G3 and G1 ($P > 0.05$).

Table 4 Effects of different feed additives on fatty acid composition, MDA content, and SOD activity in tail adipose tissue of Dorper×thin-tailed Han crossbred mutton lambs

[Table content preserved with English headers]

2.4 Effects on Perirenal Adipose Tissue Fatty Acid Composition, MDA Content, and SOD Activity

As shown in Table 5, 21 fatty acids were detected in perirenal adipose tissue. The most abundant SFA was C18:0, followed by C16:0, while C18:1cis-9 was the predominant MUFA. The major PUFA were C18:2cis-6, C20:4n-6, C18:3n-3, and eicosenoic acid (C20:1).

G2 showed significantly lower C18:0 content than G1 and G3 ($P < 0.05$), with no significant difference between G1 and G3 ($P > 0.05$). G2 exhibited significantly higher contents of C18:2cis-6, C18:3n-3, C18:3n-6, EPA, and DHA compared to G1 and G3 ($P < 0.05$). Except for C18:2cis-6 and C18:3n-6, which were significantly lower in G3 than in G1 ($P < 0.05$), no significant differences were observed for other PUFA between G1 and G3 ($P > 0.05$). G2 had significantly lower SFA content than G1 and G3 ($P < 0.05$), with no significant difference between G1 and G3 ($P > 0.05$). G2 showed significantly higher MUFA, PUFA, n-6 PUFA, n-3 PUFA contents, and PUFA/SFA ratio than G1 and G3 ($P < 0.05$). In G3, MUFA, PUFA contents, and PUFA/SFA ratio were significantly lower than in G1 ($P < 0.05$), with no significant differences for other indices ($P > 0.05$).

MDA content in G2 was significantly lower than in G1 and G3 ($P < 0.05$), with no significant difference between G3 and G1 ($P > 0.05$). Both G2 and G3 showed significantly higher SOD activity than G1 ($P < 0.05$), with G3 exhibiting significantly higher SOD activity than G2 ($P < 0.05$).

Table 5 Effects of different feed additives on fatty acid composition, MDA content, and SOD activity in perirenal adipose tissue of Dorper×thin-tailed Han crossbred mutton lambs

[Table content preserved with English headers]

2.5 Correlations Between MDA Content, SOD Activity, and PUFA Deposition

Multiple regression analysis revealed linear relationships between MDA content, SOD activity, and PUFA deposition in perirenal adipose tissue ($R^2 = 0.992$, $P < 0.001$), abdominal subcutaneous adipose tissue ($R^2 = 0.967$, $P < 0.001$), and tail adipose tissue ($R^2 = 0.965$, $P < 0.001$). MDA contributed negatively while

SOD contributed positively to the regression equations (Figure 1 [Figure 1: see original paper]).

Figure 1 Relationships between MDA content, SOD activity, and PUFA deposition in perirenal (A), abdominal subcutaneous (B), and tail adipose tissue (C) of Dorper×thin-tailed Han crossbred mutton lambs

[Figure caption preserved]

3.1 Effects on Growth Performance

Improving production performance and reducing feed-to-gain ratio to lower feed costs are urgent challenges in intensive mutton sheep farming. Lu [21] reported that dietary supplementation with different levels of freeze-dried *Allium mongolicum* powder significantly increased daily gain and dressing percentage in crossbred finishing sheep. Jia et al. [22] found that compound biological agents significantly improved average daily gain and reduced feed-to-gain ratio in Dorper×thin-tailed Han crossbred lambs. Mirzaei-Alamouti et al. [23] showed that monensin had no significant effect on dry matter intake in lambs. Our results indicate that both *Allium mongolicum* powder and microbial fermented feed had no significant effect on average daily feed intake, suggesting they did not affect diet palatability but significantly improved growth performance. The probable mechanism is that bioactive compounds in *Allium mongolicum* and probiotics in fermented feed reduce harmful gut bacteria while increasing cellulolytic and hemicellulolytic bacteria colonization in the rumen and intestine, inhibiting harmful bacteria proliferation and improving rumen microflora to enhance feed conversion efficiency.

3.2 Effects on Fatty Acid Composition in Different Fat Depots

Fatty acid composition in mutton adipose tissue is a critical indicator affecting tenderness, juiciness, flavor, edible quality, and nutritional value [24]. Research indicates that C18:0 content is closely related to mutton odor, particularly in subcutaneous fat, where high C18:0 levels intensify the characteristic odor and reduce consumer acceptance [25]. *Allium mongolicum* flavonoids have been reported to effectively reduce C18:0 content in mutton, suggesting their potential to improve odor [26]. Our study yielded similar results, likely because active compounds in *Allium mongolicum* affect enzymes involved in fat metabolism, thereby influencing synthesis of odor-related fatty acids. G2 showed significantly lower C18:0 content than G1 and G3 in all three fat depots, while G3 also had lower C18:0 than G1, though not significantly, indicating differential effects of the two additives on mutton flavor.

Linoleic acid (C18:2cis-6) is an important PUFA in mutton and a CLA precursor with health benefits including anti-atherosclerotic and lipid-lowering effects [27]. Studies show that *Allium mongolicum* aqueous and ethanol extracts significantly

increased C18:2cis-6 content in subcutaneous fat of Mongolian wether sheep [28]. Our results demonstrate that G2 not only increased C18:2cis-6 in subcutaneous fat but also in tail and perirenal fat compared to G1. Conversely, G3 significantly decreased C18:2cis-6 in perirenal fat with no significant changes in other depots.

α -Linolenic acid (C18:3n-3) is a precursor for EPA, docosapentaenoic acid (DPA, C22:5n-3), and DHA—essential n-3 fatty acids that cannot be synthesized by humans or animals and must be obtained from diet [29]. Li et al. [30] identified 14 fatty acids in *Allium mongolicum*, with high contents of C16:0, C18:2cis-6, and C18:3n-3. Our study confirmed C18:3n-3 as a major PUFA component, with G2 showing the highest C18:3n-3 content across all fat depots, likely due to direct deposition from *Allium mongolicum*. However, G3 showed no significant changes in C18:3n-3 content compared to G1, consistent with Wang [19].

EPA and DHA are crucial PUFA for human health; EPA promotes SFA metabolism and prevents atherosclerosis, while DHA supports retinal and brain development in infants [31]. Zhao et al. [32] found that *Allium mongolicum* combined with oil seeds significantly increased EPA content in Mongolian wether sheep fat, consistent with our results showing that *Allium mongolicum* powder supplementation significantly increased DHA content in abdominal subcutaneous and tail fat. High SFA intake may cause coronary heart disease, but a PUFA/SFA ratio above 0.4 can reduce circulating lipids, lower plasma cholesterol, and decrease cardiovascular disease risk [33-34]. G2 showed significantly lower SFA and higher PUFA/SFA ratios (>0.4) across all depots, while G3 had significantly lower PUFA/SFA ratios than G2 (<0.4) and even lower than G1 in perirenal fat. *Allium mongolicum* contains various bioactive compounds with strong antioxidant properties that may protect PUFA from ruminal biohydrogenation, allowing more PUFA to bypass the rumen for absorption and deposition, potentially explaining the increased PUFA/SFA ratio in G2. In conclusion, *Allium mongolicum* powder effectively improves fatty acid composition and fat quality in Dorper \times thin-tailed Han crossbred lambs, while microbial fermented feed shows limited effects on fatty acid composition.

3.3 Effects on MDA Content and SOD Activity in Different Fat Depots

During metabolism, oxygen radicals produced through enzymatic and non-enzymatic systems attack PUFA in biological membranes, initiating lipid peroxidation and forming lipid peroxides. MDA is a major end-product of membrane lipid peroxidation, and its content indirectly reflects lipid peroxidation extent [35]. SOD plays a crucial role in maintaining oxidative balance by scavenging superoxide anion radicals to protect cells from damage [36]. Jiang [37] reported that lycopene supplementation in Bamei mutton sheep significantly reduced muscle MDA content while increasing SOD activity, consistent with our findings. Wang et al. [38] found that antibiotic-free microbial fermented feed significantly increased SOD activity in piglet blood, enhancing free radical

scavenging capacity and stress resistance. In our study, G2 showed significantly reduced MDA content and increased SOD activity across all three fat depots compared to G1. G3 also significantly increased SOD activity and decreased MDA content in perirenal and abdominal subcutaneous fat, though tail fat SOD activity remained unchanged. Both additives improved fat oxidative stability, likely due to the antioxidant activity of probiotics in fermented feed [39] and flavonoids, polysaccharides, and terpenoids in *Allium mongolicum* [40]. These compounds act as reductants, blockers, and chelators at different lipid oxidation stages, terminating chain reactions, reducing reaction rates, or capturing free radicals to protect adipocytes [41].

3.4 Relationship Between PUFA Deposition and Fat Oxidative Performance

As preferred substrates for lipid oxidation, PUFA play a critical role in meat lipid oxidation [42]. Their high phospholipid affinity makes them susceptible to enzymatic oxidation, primarily involving C20:4n-6 and C18:2cis-6 [43]. Lipid oxidation during storage produces off-flavors and reduces nutritional value [44]. Lee et al. [45] found that MDA and other aldehyde oxidation products increased significantly during sausage processing while PUFA content decreased. Multiple regression analysis in Ujimqin sheep longissimus dorsi muscle showed negative contributions of MDA and glutathione peroxidase (GSH-Px) and positive contribution of SOD to the equation [46], similar to our results. Our findings demonstrate that PUFA deposition in lamb fat is closely related to lipid peroxide MDA content and antioxidant SOD activity. Reducing MDA content and increasing SOD activity can enhance PUFA deposition. Dietary supplementation with *Allium mongolicum* powder and microbial fermented feed effectively reduced MDA content in all fat depots, indicating superior lipid antioxidant performance and extended shelf life potential.

4 Conclusion

1. Dietary supplementation with *Allium mongolicum* Regel powder and microbial fermented feed increased average daily gain and reduced feed-to-gain ratio, thereby improving growth performance in housed Dorper×thin-tailed Han crossbred mutton lambs.
2. *Allium mongolicum* Regel powder supplementation effectively improved fatty acid composition and oxidative stability of body fat, enhancing fat quality in housed Dorper×thin-tailed Han crossbred mutton lambs.
3. Microbial fermented feed supplementation showed limited effects on improving fatty acid composition but enhanced oxidative stability of body fat in housed Dorper×thin-tailed Han crossbred mutton lambs.

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