Study on the Effects of Bile Salts on Production Performance and Lipid Metabolism of Simmental Crossbred Cattle

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Abstract. This experiment aimed to investigate the effects of supplementing different doses of bile salts in the diet on the production performance, blood biochemical indicators, and antioxidant capacity of Simmental crossbred cattle. Eighty healthy Simmental crossbred bulls with similar body weight and age were selected and randomly divided into four groups under identical feeding conditions: a control group and three experimental groups (I, II, III), with 20 bulls per group, comprising 10 replicates of 2 bulls each. The diets were supplemented with 0, 15, 25, and 35 mg/kg DM of bile salts, respectively, while maintaining equivalent net energy for gain and crude protein levels. After a 10-day pre-trial period and a 30-day formal trial period (40 days total), the average daily gain (ADG), feed intake (FI), blood indicators, blood biochemical indicators, and antioxidant indicators were determined for each group. The results showed that dry matter intake increased in all supplemented groups compared to the control, peaking in the 25 mg/kg group. The ADG was highest in the 25 mg/kg group, showing a 9.09% increase compared to the control group, although the difference was not statistically significant ($P > 0.05$). Dietary supplementation with 25–35 mg/kg of bile salts improved the lipid metabolism status of beef cattle and showed a positive regulatory trend on production performance. Regarding blood indicators, triglyceride and total cholesterol levels in the 35 mg/kg group were significantly lower than those in the control group ($P < 0.05$), and bile acid levels showed an upward trend across all supplemented groups. No significant differences were observed in the antioxidant indicators among the groups ($P > 0.05$). The results indicate that dietary supplementation with 25–35 mg/kg of bile salts can improve the lipid metabolism status of beef cattle and exhibits a positive regulatory trend on production performance.

Keywords: bile salts, beef cattle, production performance, blood indicators, lipid metabolism.

1. Introduction

Bile acids, as the end products of cholesterol metabolism, not only participate in lipid digestion and absorption but have also been recognized in recent years as important signaling molecules regulating energy metabolism, glucose homeostasis, and immune response. By activating pathways such as the farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (TGR5) [1], they play regulatory roles in lipid synthesis, energy expenditure, and other aspects. In livestock production, exogenous supplementation of bile acids or their derivatives has been confirmed to improve feed utilization efficiency, growth performance, and body health in various animals [2-3].

In ruminants, preliminary studies have also shown promising prospects. For example, adding bile acids to the diet of dairy cows can increase the proportion of rumen volatile fatty acids, improve the structure of the gastrointestinal microbiota, and show a tendency to increase dry matter intake [4-5].

Furthermore, industrial trials have indicated that adding bile acids to the diet of beef cattle helps improve body conformation and fat distribution.

Bile salts, as a feed additive, are used in accordance with the Ministry of Agriculture of the People's Republic of China Announcement No. 2045 "Catalogue of Feed Additive Varieties (2013)", in which "bile acids" are listed as a permitted feed additive (No. 7) [6].

However, current research on the application effects of bile salts in beef cattle, especially Simmental crossbred cattle, remains relatively limited. Therefore, this study takes Simmental crossbred cattle as the subject to investigate the effects of graded dietary supplementation of bile salts on their growth performance, blood lipid metabolism indicators, and antioxidant capacity, aiming to provide a basis for the rational application of bile salts in beef cattle production.

2. Materials and Methods

2.1. Experimental Materials

The bile salts used in the experiment were purchased from Chengdu Xinheng Pharmaceutical Co., Ltd. Their main component is bile salts (calculated as sodium cholate), with a bile salt content of 10% and microcrystalline cellulose as the carrier. The product quality complies with the provisions of "Feed Additive Part 13: Other Bile Acids" (GB 7300.1301-2025). The dosage added to the diet was calculated as pure bile salts, namely control group (0 mg/kg DM), experimental group I (15 mg/kg DM), experimental group II (25 mg/kg DM), and experimental group III (35 mg/kg DM). This experiment was conducted at the cattle farm of Shandong Shangdu Hengchang Animal Husbandry Co., Ltd.; 80 healthy Simmental crossbred cattle (♂: Simmental × ♀: Luxi Yellow cattle) with similar body weight and similar age were selected as experimental subjects. Under the same feeding conditions, they were randomly divided into four groups, namely control group, experimental group I, experimental group II, and experimental group III, with 20 cattle in each group, 10 replicates per group, and two cattle per replicate.

2.2. Experimental Design and Experimental Diets

The dosages added in this experiment referred to the common addition range of bile acids in ruminant-related studies [7,2] (e.g., 0.04%), and according to the requirements of gradient dose design in the "Guidelines for the Evaluation of Efficacy of Feeds and Feed Additives on Target Animals of Livestock and Poultry" [8], four gradients were set: 0 (control group), 15 (experimental group I), 25 (experimental group II), and 35 mg/kg (experimental group III) DM, aiming to explore the appropriate addition dosage of bile salts in the diet of Simmental crossbred cattle. Under the condition of consistent nutritional levels of the basal diet, the effects of different doses of bile salts on beef cattle were studied. The pre-trial period was 10 days for adaptation to the environment and basal diet, and the formal period was 30 days for formal treatment and data collection.

Table 1. Composition and nutrient content of the experimental diet

Ingredient	Fresh basis (%)	Nutrient composition	Content
Corn	15.54	Dry Matter(%)	55.9
Premix	1.54	Crude Protein(%)	13.45
Palm kernel meal	7.43	Ether Extract(%)	4.15
Corn germ meal	7.95	Neutral Detergent Fiber(%)	35.38
Soybean meal	4.45	Acid Detergent Fiber(%)	18.32
Salt	0.27	Ash (%)	7.85
Buffer	0.76	Net Energy for Gain(Mcal/kg)	1.16
Distiller’s grains	16.99		
Silage	40.70		
Hay	4.36		

Note: Per kilogram of premix provides: VA 190,000 IU; VD_3 3,000 IU; VE 5,000 IU; Fe 4,000 mg; Mn 400 mg; Zn 2,000 mg; Cu 800 mg; I 40 mg; Se 13 mg; Ca 150 mg; P 40 mg. Nutrient indicators are measured values; net energy for gain is a calculated value, calculated using the Cargill BeefMAX formulation system for beef cattle, based on the NRC (2000) Nutrient Requirements of Beef Cattle, Eighth Edition model.

2.3. Feeding Management

The experimental beef cattle were all kept in a loose housing system. Before numbering, they were all vaccinated against foot-and-mouth disease and underwent internal and external deworming. The initial body weight of the experimental cattle was (410 ± 25) kg, and the age was (12 ± 1) months. However, to ensure the independence of the replicate units, the cattle in each group were assigned to 10 independent pens (each pen approximately 20 square meters), with two cattle per pen, and physical barriers were placed between pens. The experimental cattle were fed twice daily (07:00 and 17:00) using a uniform total mixed ration (TMR) feeding mode, while ensuring that the feed intake and drinking behavior of each replicate unit were not affected by other units.

2.4. Experimental Samples and Nutritional Analysis of TMR Diet

Dry matter (DM) content was determined by oven drying method; crude protein (CP) was determined by Kjeldahl method; crude ash (Ash) was determined by ashing method; neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the Van Soest detergent fiber method; net energy for gain was calculated using the Cargill BeefMAX formulation system for beef cattle, and the nutritional standards were based on the NRC (2000) *Nutrient Requirements of Beef Cattle*, Eighth Edition model.

2.5. Growth Performance Measurement

On the first day of the formal trial period, the experimental cattle were weighed on an empty stomach before the morning feeding, recorded as initial body weight (IBW). On the first day after the end of the formal trial period, before the morning feeding, the experimental cattle were weighed on an empty stomach, recorded as final body weight (FBW), and the average daily gain (ADG) during the formal trial period was calculated. During the formal trial period, before the morning feeding on the next day, the remaining feed was collected and weighed. DMI (kg) = daily feed intake × dietary dry matter content, and dry matter intake (DMI) was calculated. Combined with daily DMI and ADG, the feed conversion ratio (G/F = ADG/DMI) was calculated.

2.6. Determination of Blood Biochemical Indices

One day before the end of the formal experiment, blood was collected from the tail root vein before morning feeding. After standing for 1 to 2 hours, the blood was centrifuged at 3400 r/min for 15 minutes to separate the serum. The upper serum layer was aspirated using a pipette, aliquoted into capped 0.5 ml centrifuge tubes, and stored at -20°C until analysis.

Glucose (GLU), total bile acids (TBA), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), total protein (TP), urea nitrogen (UN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using an automatic biochemical analyzer.

The serum levels of total antioxidant capacity (T-AOC), superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) were measured by ELISA. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

2.7. Statistical Analysis

The experimental data were preliminarily organized using Excel 2007. Statistical analysis was performed using SPSS 18.0, with pen replicate as the statistical unit (10 replicates per group, each replicate corresponding to two cattle in one independent pen). One-way analysis of variance (ONE-Way ANOVA) was conducted, and analysis of covariance (ANCOVA) with initial body weight as a covariate was also used. Both ANOVA and ANCOVA used pen replicate as the statistical unit. Although the loose housing environment may have a slight risk of environmental sharing, physical isolation measures ensured the independence of replicate units to avoid pseudoreplication. Duncan's multiple range test was performed. Results are presented as mean ± standard error of the mean (SEM). The significance levels were set as follows: $p > 0.05$ indicated no significant difference, $p < 0.05$ indicated a significant difference, and $p < 0.01$ indicated an extremely significant difference.

3. Results

3.1. Effects of Different Supplemental Levels on Growth Performance of Beef Cattle

Table 2. Effects of different supplemental levels on growth performance of beef cattle

Item	Group			
	Control group	Experimental group I	Experimental group II	Experimental group III
Initial body weight (IBW) kg	412.39±27[a]	421.5±22[a]	418±23.25[a]	425.57±28.65[a]
Final body weight (FBW) kg	455±22.77[a]	466±25.16[a]	465±26.32[a]	469.35±30.77[a]
Average daily gain (ADG) kg/d	1.43±0.29[a]	1.5±0.33[a]	1.56±0.41[a]	1.48±0.36[a]
Average dry matter intake (ADMI) kg/d	11.53±0.24[a]	11.94±0.26[a]	12.23±0.35[a]	12.15±0.88[a]
Feed-to-gain ratio (F/G)	8.06±0.36[a]	7.96±0.29[a]	7.84±0.27[a]	8.21±0.29[a]

Note: Within the same indicator, the ANCOVA analysis results were consistent with the ANOVA conclusions. Different lowercase superscript letters in the same row indicate a significant difference ($P < 0.05$); different uppercase superscript letters indicate an extremely significant difference ($P < 0.01$); the same superscript letter or no superscript indicates no significant difference ($P > 0.05$).

As shown in Table 2, the average dry matter intake (ADMI) of all experimental groups was higher than that of the control group, with the highest intake observed in experimental group II (25 mg/kg), which was 6.10% higher than that of the control group. Although the ADMI of experimental group III (35 mg/kg) was slightly lower than that of experimental group II, it remained higher than that of the control group. The trend of average daily gain (ADG) was generally consistent with that of feed intake, reaching its maximum value in experimental group II (1.56 ± 0.41 kg/d), which was 9.09% higher than that of the control group (1.43 ± 0.29 kg/d). The feed to gain ratio (F/G) was the lowest in experimental group II, which was 2.72% lower than that of the control group. Although experimental group II showed certain advantages in ADMI, ADG, and F/G, the differences among all groups did not reach a significant level ($P > 0.05$). Notably, the standard error of ADG in experimental group II (±0.41) was higher than that of the control group (±0.29), indicating greater individual variation in weight gain within this group, which may be an important reason for the lack of significant differences among groups.

3.2. Effects of Different Supplemental Levels on Blood Biochemical Indices of Beef Cattle

Table 3. Effects of different supplemental levels on blood indices of beef cattle

Item	Group			
	Control group	Experimental group I	Experimental group II	Experimental group III
Glucose GLU/(mmol/L)	2.35±0.35^{a}	2.33±0.27^{a}	2.36±0.31^{a}	2.30±0.37^{a}
Total bile acids TBA/(μmol/L)	8.52±1.43^{a}	8.63±2.15^{a}	8.92±1.67^{a}	9.22±1.92^{a}
Triglycerides TG/(mmol/L)	0.45±0.12^{a}	0.41±0.21^{ab}	0.39±0.12^{ab}	0.35±0.11^{b}
Total cholesterol TC/(mmol/L)	2.99±0.22^{a}	2.89±0.32^{ab}	2.77±0.24^{ab}	2.62±0.25^{b}
High-density lipoprotein HDL/(mmol/L)	1.63±0.35^{a}	1.74±0.21^{a}	1.78±0.31^{a}	1.76±0.26^{a}
Low-density lipoprotein LDL/(mmol/L)	0.83±0.36^{a}	0.78±0.28^{a}	0.82±0.32^{a}	0.81±0.26^{a}
Very low-density lipoprotein VLDL/(mmol/L)	0.64±0.26^{a}	0.60±0.28^{a}	0.51±0.27^{a}	0.54±0.21^{a}
Total protein TP/(g/L)	17.31±0.65^{a}	17.34±0.49^{a}	17.28±0.51^{a}	17.33±0.52^{a}
Alanine aminotransferase ALT (U/L)	31.49±6.81^{a}	31.78±8.43^{a}	33.50±8.21^{a}	34.76±7.28^{a}
Aspartate aminotransferase AST (U/L)	56.21±12.71^{a}	55.52±11.74^{a}	53.27±17.41^{a}	56.76±11.28^{a}
Urea nitrogen UN (mmol/L)	2.72±0.38^{a}	2.77±0.23^{a}	2.75±0.31^{a}	2.81±0.25^{a}

Note: Different lowercase superscript letters in the same row indicate a significant difference ($P < 0.05$); different uppercase superscript letters indicate an extremely significant difference ($P < 0.01$); the same superscript letter or no superscript indicates no significant difference ($P > 0.05$).

As shown in Table 3, for triglycerides: the levels in each experimental group showed significant differences among different treatment groups ($P < 0.05$). Specifically, the TG concentration in experimental group III (35 mg/kg) was significantly lower than that in the control group ($P < 0.05$), and was also significantly lower than that in experimental groups I and II ($P < 0.05$). No significant differences were observed among experimental group I, experimental group II, and the control group ($P > 0.05$). For bile acids: serum TBA levels in each experimental group showed no significant difference compared with the control group ($P > 0.05$). Numerically, there was a tendency to increase with increasing supplementation level, but this change did not reach statistical significance. For total cholesterol: the change pattern in each experimental group was completely consistent with that of TG. Compared with the experimental groups, the control group showed a decreasing trend in blood total cholesterol. The blood cholesterol content in the control group was 9.93% higher than that in experimental group III, and the difference was significant ($P < 0.05$). For urea nitrogen: compared with the experimental groups, the control group showed an increasing trend in blood urea nitrogen, but the differences among groups were not significant ($P > 0.05$).

3.3. Effects of Different Supplemental Levels on Blood Antioxidant Indices of Beef Cattle

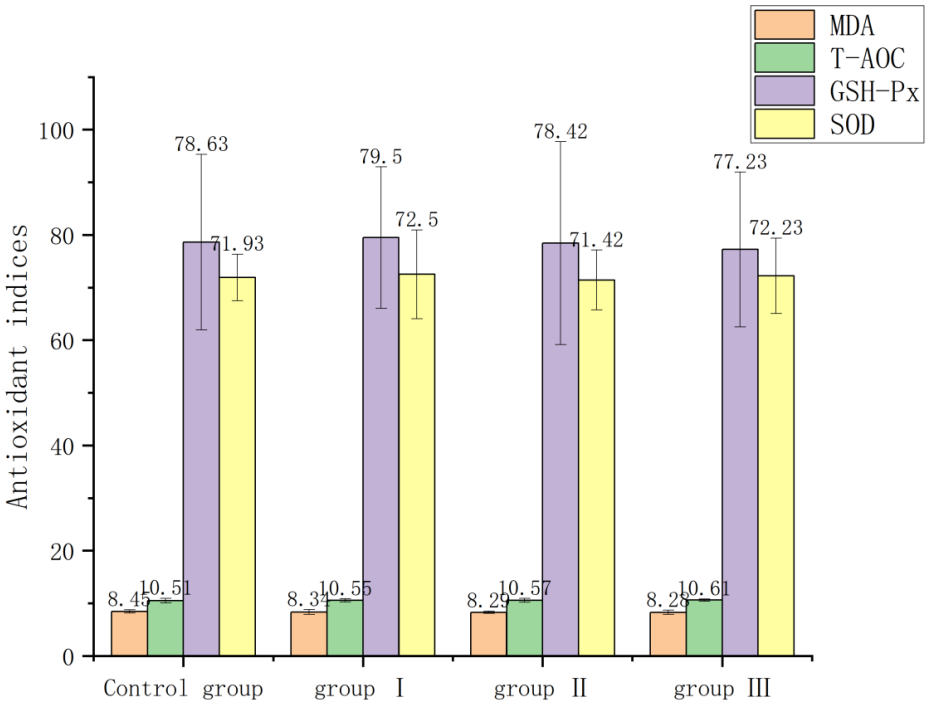

Figure 1. Effects of different bile salt supplemental levels on serum antioxidant indices of beef cattle

As shown in Figure 1, malondialdehyde: all experimental groups were lower than the control group, but the differences among groups were not significant ($P > 0.05$); total antioxidant capacity: experimental group III had the highest value, but the differences among groups were not significant ($P > 0.05$).3 Discussion

4. Discussion

4.1. Effects of different supplemental levels on production performance of beef cattle

In this study, the production performance indicators did not show a simple linear increase with increasing bile salt supplementation levels, but instead exhibited a non-monotonic dose-response relationship. When the dietary supplementation level was 25 mg/kg, beef cattle achieved the best daily gain and feed intake. This finding is consistent with the results of Zhou et al. [2] in finishing pigs. It is worth noting that although not statistically significant, this consistent trend suggests that bile salts may have a similar mechanism of promoting feed intake across different species. The underlying mechanism may be related to exogenous bile salts stimulating bile secretion, thereby optimizing the rumen fermentation environment and nutrient digestion efficiency. Fan et al. [4] found that adding bile salts to the diet of dairy cows could increase the proportion of rumen volatile fatty acids and improve the number of beneficial microbiota in the digestive tract. Chen et al. reported that adding bile acids tended to increase dry matter intake in mid-lactation dairy cows [5]. The results of this experiment are similar to the above reports. When the dose was increased to 35 mg/kg, although feed intake remained high, the gain efficiency decreased. This phenomenon reveals the optimal dose window characteristic of bile salts. The potential mechanism may be that an appropriate dose of bile salts optimizes energy metabolism by promoting lipid digestion and absorption and acting as a signaling molecule; however, excessive addition may interfere with the balance of the endogenous bile acid pool or cause slight feedback inhibition on feeding or metabolism through pathways such as the farnesoid X receptor (FXR) [9,10]. Therefore, compared with 35 mg/kg, 25 mg/kg showed higher efficiency in promoting growth, providing a key basis for determining the most cost-effective supplemental dose in production.

4.2. Effects of different supplemental levels on blood biochemical indices of beef cattle

Bile acids, as important signaling molecules, are widely involved in metabolic regulation by activating nuclear receptors such as the farnesoid X receptor (FXR) [1]. In this study, although serum total bile acid levels did not change significantly after exogenous bile salt supplementation, they showed a certain increasing tendency numerically ($P > 0.05$), suggesting that exogenous supplementation may directly or indirectly (through feedback regulation) expand the endogenous bile acid pool. In terms of lipid metabolism, this effect is consistent with classical mechanisms: for example, Watanabe et al. [10] pointed out that bile acids can inhibit SREBP-1c expression through the FXR/SHP pathway, thereby reducing triglyceride (TG) synthesis; the simultaneous reduction in TG and total cholesterol (TC) observed in beef cattle in this study is indirect evidence of activation of this pathway. This finding echoes the conclusions of improved lipid metabolism observed by Li et al. [11] in dairy cows and by Dicks et al. [13] in dairy cows fed high-concentrate diets, indicating that this mechanism may be conserved in ruminants. Furthermore, the decreasing trend of total cholesterol (TC) in this study is consistent with the classical theory that bile acids can feedback inhibit the activity of cholesterol 7α-hydroxylase (CYP7A1) by activating the farnesoid X receptor (FXR), thereby regulating the rate of conversion of cholesterol to bile acids to maintain homeostasis [12], further supporting the rationale for bile salts improving lipid metabolism in beef cattle.

It is worth noting that the significant reductions in triglycerides (TG) and total cholesterol (TC) in this study only occurred in the high-dose group (35 mg/kg), which is consistent with the view proposed by Thomas et al.[14] that the lipid-lowering effect of bile acids has a threshold effect [4]. This dose-response relationship provides an important reference for practical application. In terms of safety, Du et al., in a study on the digestive metabolism of dairy cows with fatty liver, indicated that excessive bile salts may cause liver damage [15], while alanine aminotransferase (ALT) and aspartate aminotransferase (AST) can indicate the degree of liver damage [16]. In this experiment, there were no significant differences in ALT and AST values among groups ($P > 0.05$), indicating that the supplemental doses used did not cause liver damage to the animals. In addition, although bile cannot directly participate in protein digestion, it can improve the intestinal environment and play an auxiliary synergistic role. The chyme in the abomasum is strongly acidic, and trypsin has optimal activity under weak alkaline conditions. The bicarbonate present in bile, upon entering the duodenum, improves the intestinal pH environment, ensuring the effectiveness of trypsin [17]. Blood urea nitrogen (BUN) is a key indicator of protein status and rumen production performance in animals [18]. In this study, blood urea nitrogen levels were not affected by the diet, indicating that protein metabolic status remained stable.

It is worth noting that the dose gradient design (0, 15, 25, 35 mg/kg) of this study, while confirming the biological effects of bile salts, also reflects the complexity of their action. The response patterns of production performance and lipid metabolism indicators to dose increments were not entirely consistent: average daily gain reached a plateau at 25 mg/kg, whereas significant improvement in blood lipid parameters occurred at 35 mg/kg. This difference suggests that bile salts may act through multiple mechanisms, and the activation thresholds for different physiological functions may vary. Although no finer gradient was set between 0 and 15 mg/kg, making it difficult to precisely define the minimum effective threshold, the existing gradient design is sufficient to reveal the dose-dependent trend of bile salt supplementation and its significant regulatory effect on lipid metabolism.

4.3. Effects of different supplemental levels on blood antioxidant indices of beef cattle

Blood antioxidant indices are important bases for evaluating the body's antioxidant status [19-20]. In this study, the malondialdehyde (MDA) content in all experimental groups showed a decreasing trend compared with the control group, although the differences among groups did not reach statistical significance ($P > 0.05$). As the end product of lipid peroxidation, MDA is a sensitive marker for evaluating oxidative damage [21]. The decreasing trend in its content may suggest that bile salt supplementation helps reduce the accumulation of lipid peroxidation products in the body [22]. In nutritional intervention studies, such consistent biological trends have important indicative value [23].

The underlying mechanism may be related to the activation of endogenous antioxidant signaling pathways by bile acids [24], thereby alleviating oxidative damage.

Total antioxidant capacity (T-AOC) is an important indicator for measuring the overall antioxidant level of the body, reflecting the body's defense ability against oxidative stress [25]. The data from this study showed that experimental group III (35 mg/kg) had the highest T-AOC value, which was 0.95% higher than that of the control group, and experimental groups I and II also showed a slight increase. Although the differences among groups were not significant, this increasing trend has positive biological significance. This may be attributed to the role of bile salts in promoting the absorption of fat-soluble vitamins (such as vitamin E). Studies by Ridlon [26] and Brzezinska-Slebodzinska et al. [27] have shown that bile acids play a key role in the absorption of fat-soluble vitamins, and vitamin E itself is an important antioxidant. By stimulating bile secretion, bile salts optimize the gastrointestinal absorption environment, not only improving the utilization of conventional nutrients but also potentially enhancing the absorption of antioxidant nutrients such as vitamin E, thereby providing a material basis for improving the body's total antioxidant capacity.

Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are important antioxidant enzymes in the body; the former scavenges superoxide anion radicals, and the latter decomposes peroxides such as hydrogen peroxide [28]. In this experiment, the activities of SOD and GSH-Px in each supplementation group showed no significant differences compared with the control group ($P > 0.05$). This may be related to the relatively short experimental period, as the response of blood antioxidant enzyme indicators to nutritional intervention may require a longer time to become fully significant[17]; it may also indicate that under the conditions of this experiment, the beef cattle's own antioxidant enzyme system was already able to maintain homeostasis, and the supplementation of exogenous bile salts did not produce further additive effects.

5. Conclusion

The results of this study indicate that dietary supplementation with bile salts exerts positive regulatory effects on the growth performance and lipid metabolism status of Simmental crossbred beef cattle. Comprehensive evaluation of production performance indicators revealed that the 25 mg/kg DM supplementation group showed the best improving trends in average daily gain (1.56 ± 0.41 kg/d) and feed intake (12.23 ± 0.35 kg/d), with ADG increased by 9.09% and F/G decreased by 2.72% compared with the control group. Although the data in this group did not reach statistical significance ($P > 0.05$), combined with its comparative performance against other dose groups, it suggests that 25 mg/kg DM may be a potential optimal dose for improving production performance. In terms of lipid metabolism, the 35 mg/kg DM dose exhibited more definite biological effects, significantly reducing serum triglyceride (-22.2%) and total cholesterol (-9.93%) levels ($P < 0.05$). Serum bile acid levels in all supplementation groups showed an increasing trend, and liver function indicators (ALT, AST) and protein metabolism indicators (UN) did not show abnormal fluctuations, confirming the safety of the tested doses. It should be noted that the experimental period was relatively short and the sample size was limited, and some indicators did not reach statistical significance. However, the results of this study indicate that dietary supplementation with 25-35 mg/kg of bile salts exerts positive regulatory trends on the growth performance and lipid metabolism status of Simmental crossbred beef cattle. Comprehensive evaluation suggests that dietary supplementation with 25 mg/kg of bile salts provides a scientific basis and data reference for the rational application of bile salts as a functional feed additive in beef cattle production.

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