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Effects of Rumen-Protected Betaine and Rumen-Protected Choline on Growth Performance, Digestive Performance, Slaughter Performance, and Fat Deposition in 1- to 3-Month-Old Hu Sheep (Postprint)

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Abstract

This experiment aimed to investigate the effects of rumen-protected betaine (RPB) and rumen-protected choline (RPC) on growth performance, digestive performance, slaughter performance, and fat deposition in 1- to 3-month-old Hu sheep. Thirty male Hu lambs of similar body weight at approximately 1 month of age were selected and randomly divided into three groups: a control group, an RPB group, and an RPC group, with 10 lambs per group. The control group was fed a basal diet, while the RPB and RPC groups were fed the basal diet supplemented with 2.9 g/kg RPB and 2.5 g/kg RPC on a dry matter (DM) basis, respectively. The preliminary period lasted 21 days, and the formal experimental period lasted 54 days. The results showed that: 1) Dietary supplementation with RPB and RPC had no significant effects on average daily gain (ADG), average daily feed intake (ADFI), or feed conversion ratio (FCR) in 1- to 3-month-old Hu sheep ($P>0.05$), but the ADG of the RPB and RPC groups increased by 19.97% and 27.75% compared with the control group, respectively. 2) Dietary supplementation with RPB and RPC had no significant effects on the apparent digestibility of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), DM, or organic matter (OM) in 1- to 3-month-old Hu sheep ($P>0.05$). 3) Dietary supplementation with RPB and RPC significantly reduced backfat thickness in 1- to 3-month-old Hu sheep ($P<0.05$), and had no significant effects on pre-slaughter live weight, dressing percentage, carcass weight, GR value, loin eye area, abdominal fat weight, perirenal fat weight, or intramuscular fat (IMF) content ($P>0.05$). However, the loin eye area of the RPB and RPC groups increased by 10.86% and 3.16% compared with the control group, respectively; IMF content increased by 17.27% and 36.36%, respectively; and perirenal fat weight decreased by 20.60% and 22.67%, respectively. Based on

these results, it was concluded that dietary supplementation with either 2.9 g/kg RPB or 2.5 g/kg RPC can improve fat deposition sites and carcass quality in 1- to 3-month-old Hu sheep, and that the two additives had comparable effects on digestive performance, growth performance, and slaughter performance under dietary nutrient levels that met maintenance requirements.

Full Text

Effects of Rumen-Protected Betaine and Rumen-Protected Choline on Growth Performance, Digestive Performance, Slaughter Performance, and Fat Deposition in 1- to 3-Month-Old Hu Lambs

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Abstract

This study investigated the effects of rumen-protected betaine (RPB) and rumen-protected choline (RPC) on growth performance, digestive performance, slaughter performance, and fat deposition in 1- to 3-month-old Hu lambs. Thirty approximately 1-month-old Hu male lambs with similar body weight were randomly divided into three groups (n = 10): control, RPB, and RPC. The control group received a basal diet, while the RPB and RPC groups received the basal diet supplemented with 2.9 g/kg RPB and 2.5 g/kg RPC (on a dry matter basis), respectively. The experiment consisted of a 21-day pre-trial period followed by a 54-day formal trial period. The results showed that: (1) dietary supplementation with RPB and RPC had no significant effects on average daily gain, average daily feed intake, or feed-to-gain ratio in 1- to 3-month-old Hu lambs ($P > 0.05$), although average daily gain increased by 19.97% and 27.75% in the RPB and RPC groups, respectively, compared with the control; (2) supplementation with RPB and RPC did not significantly affect the apparent digestibility of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter (DM), or organic matter (OM) ($P > 0.05$); and (3) RPB and RPC supplementation significantly reduced backfat thickness ($P < 0.05$) but had no significant effects on live weight before slaughter, dressing percentage, carcass weight, GR value, loin eye area, abdominal fat weight, perirenal fat weight, or intramuscular fat (IMF) content ($P > 0.05$). However, compared with the control group, loin eye area increased by 10.86% and 3.16%, IMF content increased by 17.27% and 36.36%, and perirenal fat weight decreased by 20.60% and 22.67% in the RPB and RPC groups, respectively. These findings indicate that supplementation with either 2.9 g/kg RPB or 2.5 g/kg RPC can improve fat deposition patterns and carcass quality in 1- to 3-month-old Hu lambs, with both additives demonstrating comparable

effects under dietary nutrient levels that meet maintenance requirements.

Keywords: rumen-protected betaine; rumen-protected choline; growth performance; digestive performance; slaughter performance; fat deposition

Introduction

Intensive farming practices in China have reduced physical activity in meat sheep, and the scarcity of fresh green forage has led to progressively thicker backfat and abdominal fat deposits in lambs during the late fattening period, severely compromising meat quality and economic returns. Concurrently, rising living standards have shifted consumer demand toward premium lamb meat that is tender, flavorful, and nutritious, necessitating modified fattening techniques to improve meat quality. Previous research has demonstrated that betaine (trimethylglycine) serves as a methyl donor in animals, promoting carnitine synthesis and increasing free carnitine content in the liver of finishing pigs. This facilitates the transport of long-chain fatty acids into muscle mitochondria for β-oxidation, thereby reducing subcutaneous fat while increasing intramuscular fat content. Studies have also reported that betaine supplementation reduces abdominal fat percentage and improves carcass quality in broiler chickens. Similarly, choline participates in methyl metabolism and can be oxidized twice by choline oxidase to generate betaine, thereby increasing betaine availability in animals.

However, most research on betaine and choline' s effects on fat redistribution has focused on aquatic animals and poultry, with limited reports in meat sheep. Therefore, this study examined the effects of dietary supplementation with rumen-protected betaine (RPB) and rumen-protected choline (RPC) on growth performance, digestive performance, slaughter performance, and fat deposition in 1- to 3-month-old Hu lambs, aiming to provide theoretical and practical insights for modified fattening strategies in meat sheep production.

Materials and Methods

1.1 Experimental Animals and Diets

Thirty 1-month-old Hu male lambs with an average body weight of (10.08 $\pm$ 1.16) kg were randomly assigned to three groups (n = 10) using a single-factor randomized design: control, RPB, and RPC. The control group received a basal diet formulated according to NRC (2007) requirements for sheep weighing (10.0 $\pm$ 1.1) kg with a daily gain of 100 g/d. The RPB group received the basal diet supplemented with 2.9 g/kg RPB (betaine content $\geq$ 55%, safe rumen bypass rate $\geq$ 60%, provided by Yixing Tianshi Feed Co., Ltd.), while the RPC group received the basal diet supplemented with 2.5 g/kg RPC (choline content $\geq$ 50%, safe rumen bypass rate $\geq$ 85%, provided by Ascor Chimici S.r.l., Italy). Supplementation levels were based on previous studies by Cui et al. [**?**] and Li

et al. [**?**]. The composition and nutrient levels of the basal diet are presented in Table 1 .

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

Ingredients	Content	Nutrient levels	Content
Peanut vine		ME/(MJ/kg)	
Soybean stalk		CP	
Rice straw		NDF	
Corn		ADF	
Soybean meal		Ca	
Wheat bran			
Premix			
NaCl			
Total			

*Note: 1) Each kilogram of premix contained: VA 125,000 IU, VD3 55,000 IU, VE 200 mg, niacin 350 mg, Zn 2.0 g, Cu 0.25 g, Mn 2.0 g, Se 5.5 mg, Ca 150 g, P 15 g, NaCl 75 g. 2) ME, Ca, and P were calculated values based on "Nutrition Parameters and Feeding Standard for Animals" [**?**] (2nd ed.), while other nutrient levels were measured values.*

1.2 Feeding Management

The experiment was conducted at Donglin Ecological Breeding Sheep Farm in Taicang City, Jiangsu Province. Lambs were immunized according to standard farm procedures, with routine cleaning and disinfection maintained throughout the trial. During the experimental period, concentrate and roughage were mixed and provided ad libitum at 08:00 and 16:00 daily, with additives mixed into the concentrate. Fresh water was available at all times, and lambs were housed individually. Feed intake and refusals were recorded daily. The trial consisted of a 21-day pre-experimental period followed by a 54-day formal experimental period.

1.3.1 Nutrient Apparent Digestibility Determination

On day 42 of the experiment, five lambs per group were randomly selected for a digestion-metabolism trial using the total fecal collection method. After a 3-day adaptation period, feces were collected continuously for 5 days at 12-hour intervals, weighed, and mixed with 10% dilute sulfuric acid (10% of fecal weight) for nitrogen fixation. The 5-day samples were pooled and stored at -20 °C. The contents of CP, NDF, ADF, DM, and OM in feed, refusals, and feces were determined according to methods described in "Feed Analysis and Feed Quality Detection Technology" edited by Yang Sheng [**?**]. Nutrient apparent digestibility was calculated as:

$$\text{Nutrient apparent digestibility (\%)} = \frac{\text{Intake} - \text{Excretion}}{\text{Intake}} \times 100$$

1.3.2 Growth Performance Determination

Lambs were weighed after overnight fasting on days 1 and 65 to determine initial and final body weight, respectively, and average daily gain (ADG) was calculated. Daily feed intake and refusals were recorded to calculate average daily feed intake (ADFI) and feed-to-gain ratio (F/G).

1.3.3 Slaughter Performance Determination

On day 65, three lambs per group were randomly selected, fasted for 24 h (with free access to water), and then slaughtered. Measured parameters included live weight before slaughter, carcass weight, dressing percentage, loin eye area, GR value (tissue thickness at 11 cm from the dorsal midline between the 12th and 13th ribs), backfat thickness, abdominal fat weight, and perirenal fat weight. Sampling procedures followed methods described by Zhao [**?**]. The longissimus dorsi muscle was collected, freeze-dried for 48 h, ground into powder, and analyzed for intramuscular fat (IMF) content using the Soxhlet extraction method described in reference [**?**], with results expressed as wet weight IMF content.

1.4 Data Analysis and Statistics

Data were initially processed using Excel 2013 and then subjected to one-way ANOVA using SPSS 19.0. Results are presented as means with standard errors, with $P < 0.05$ considered statistically significant.

Results

2.1 Effects of RPB and RPC on Growth Performance and Nutrient Apparent Digestibility

As shown in Table 2 , with no significant differences in initial body weight among groups ($P > 0.05$), final body weight also did not differ significantly ($P > 0.05$). Average daily gain in the RPB and RPC groups increased by 19.97% and 27.75%, respectively, compared with the control group, though differences were not statistically significant ($P > 0.05$). Neither RPB nor RPC supplementation significantly affected average daily feed intake or feed-to-gain ratio in 1- to 3-month-old Hu lambs ($P > 0.05$).

Table 2 Effects of RPB and RPC on growth performance of 1- to 3-month-old Hu lambs

Items	Control	RPB	RPC	P-value
Initial body weight (kg)				
Final body weight (kg)				

Items	Control	RPB	RPC	P-value
ADG (g/d)				
ADFI (g/d)				
F/G				

Note: In the same row, values with no letter or the same letter superscripts indicate no significant difference (P > 0.05), while different letters indicate significant difference (P < 0.05). The same applies below.

Table 3 shows that dietary supplementation with RPB and RPC did not significantly affect the apparent digestibility of CP, NDF, ADF, DM, or OM in 1- to 3-month-old Hu lambs (P > 0.05).

Table 3 Effects of RPB and RPC on nutrient apparent digestibility of 1- to 3-month-old Hu lambs

Items	Control	RPB	RPC	P-value
CP				
NDF				
ADF				
DM				
OM				

2.2 Effects of RPB and RPC on Slaughter Performance and Fat Deposition

As shown in Table 4 , no significant differences were observed among groups in live weight before slaughter or dressing percentage (P > 0.05). RPB and RPC tended to increase and decrease carcass weight, respectively, but differences were not significant (P > 0.05). Backfat thickness was significantly lower in both RPB and RPC groups compared with the control (P < 0.05). Although GR value, loin eye area, abdominal fat weight, perirenal fat weight, and IMF content did not differ significantly among groups (P > 0.05), loin eye area increased by 10.86% and 3.16%, IMF content increased by 17.27% and 36.36%, and perirenal fat weight decreased by 20.60% and 22.67% in the RPB and RPC groups, respectively, compared with the control.

Table 4 Effects of RPB and RPC on slaughter performance and fat deposition of 1- to 3-month-old Hu lambs

Items	Control	RPB	RPC	P-value
Live weight before slaughter (kg)				
Carcass weight (kg)				
Dressing percentage (%)				

Items	Control	RPB	RPC	P-value
GR value (cm)				
Loin eye area (cm^2)				
Backfat thickness (cm)	0.68a	0.36b	0.37b	
Abdominal fat weight (g)				
Perirenal fat weight (g)				
IMF content (%)				

Discussion

3.1 Effects of RPB and RPC on Growth Performance and Digestive Performance

Dietary RPC supplementation can partially replace methionine and promote animal growth [**?**, **?**]. In this study, RPC increased average daily gain in 1- to 3-month-old Hu lambs, though the effect was not statistically significant. Betaine' s methyl transfer efficiency is 12–15 times higher than that of choline, and as a methyl donor, betaine can spare choline while also promoting growth. Our results showed that both RPB and RPC increased average daily gain and improved feed utilization efficiency, but without statistical significance. These findings are consistent with previous studies by Cui et al. [**?**] and Li et al. [**?**], though the lack of significant effects may be attributed to differences in nutrient requirements at various growth stages or variations in dietary nutrient levels [**?**]. Previous reports on the effects of choline and betaine on nutrient digestibility have been inconsistent [**?**, **?**]. Theoretically, at least 60% of choline chloride and 85% of betaine should be protected from rumen microbial degradation after consumption of RPB and RPC, suggesting that these additives may not affect nutrient apparent digestibility. Our results demonstrated that RPB and RPC had no significant effects on the apparent digestibility of CP, NDF, ADF, DM, or OM in 1- to 3-month-old Hu lambs. Similar findings were reported by Tian et al. [**?**], who observed no significant effects of RPC supplementation on DM, CP, ether extract (EE), or NDF digestibility in Qianbei Ma goats, and by Liu [**?**], who found no significant effects of betaine on nutrient digestibility in finishing Hu sheep. These results support our hypothesis.

3.2.1 Effects of RPB and RPC on Slaughter Performance

Previous studies have shown that dietary betaine supplementation can improve slaughter performance in animals [**?**]. In this experiment, dietary RPB and RPC supplementation did not significantly affect pre-slaughter live weight, carcass weight, dressing percentage, GR value, or loin eye area compared with the control group, which aligns with findings by Li et al. [**?**] and Yan [**?**]. The lack of significant changes in dressing percentage may be attributed to identical basal diets and similar feed intake across groups, as feed intake directly affects dressing percentage. Although GR value and loin eye area were not sig-

nificantly affected by RPB or RPC supplementation, both showed numerical improvements compared with the control. This may be because choline can prevent methionine from being used as a methyl donor, thereby sparing methionine for protein synthesis [**?**, **?**]. However, the moderate protein level in our basal diet may have limited the magnitude of this effect. Zhao et al. [**?**] reported that higher carcass weight is associated with larger loin eye area, which is consistent with our observation that the RPB group had higher carcass weight than the control, though the RPC group showed the opposite trend, possibly due to lower methyl donor efficiency of choline compared with betaine.

3.2.2 Effects of RPB and RPC on Muscle Fat Deposition

Animal body fat deposition represents a balance between fat anabolism and catabolism [**?**]. Betaine has been reported to enhance lipolytic enzyme activity [**?**], thereby redistributing fat deposits. Additionally, betaine may regulate fat metabolism by participating in key steps of fat synthesis, breakdown, and transport [**?**]. After entering the body, some choline is synthesized into phosphatidylcholine, which promotes chylomicron synthesis in the liver and inhibits hepatic fat accumulation, thereby redistributing fat deposits. Choline also promotes fatty acid transport in the form of lecithin during fat metabolism, accelerating fat catabolism and reducing body fat deposition. A small portion of choline is converted to betaine through two-step oxidation to participate in fat metabolism.

Our results showed that compared with the control, RPB supplementation significantly reduced backfat thickness and perirenal fat weight by 20.60% while increasing IMF content by 17.27% in 1- to 3-month-old Hu lambs. These findings are consistent with reports by Martins et al. [**?**] that 0.1% dietary betaine increased IMF content in porcine longissimus dorsi muscle, and with studies by Yu et al. [**?**] and Hu et al. [**?**] demonstrating reduced backfat thickness in pigs fed betaine-supplemented diets. Similarly, RPC supplementation significantly reduced backfat thickness and perirenal fat weight by 22.67% while increasing IMF content by 36.36% compared with the control, which aligns with the trend reported by Bian et al. [**?**] that 500 mg/kg choline supplementation reduced backfat thickness in pigs. As Dikeman [**?**] noted, IMF is fat tissue distributed within muscles and around muscle fiber bundles, representing a key determinant of meat quality that is strongly correlated with flavor, tenderness, and juiciness [**?**]. Appropriate IMF content can improve meat quality. In this study, both RPB and RPC reduced backfat thickness and perirenal fat weight while increasing IMF content, thereby improving fat distribution and carcass quality in lambs and achieving modified fattening effects. However, the mechanisms underlying IMF formation in lambs require further investigation.

Conclusion

In conclusion, dietary supplementation with either 2.9 g/kg RPB or 2.5 g/kg RPC can improve fat deposition patterns and carcass quality in 1- to 3-month-

old Hu lambs, achieving modified fattening effects. Based on digestive performance, growth performance, and slaughter performance, both additives demonstrate comparable efficacy under dietary nutrient levels that meet maintenance requirements.

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