

Effects of rumen-protected choline supplied at different dietary energy levels on growth performance and meat quality of fattening goats

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ABSTRACT. The effects of rumen-protected choline (RPC) supplementation in different energy level diets on growth performance and meat guality of fattening goats were examined in this study. In total, 40 six-month-old Cashmere goats (initial body weight 24.30 ± 2.64 kg) were randomly assigned to four treatments: two different digestible energy (DE) levels (11.94 and 10.89 MJ/kg) and RPC supplement levels (0 and 0.2%). The experiment lasted for 70 days, with the first 10 days of acclimation and next 60 days of formal experimental period. It was shown that RPC supplementation in a low-energy diet improved the overall average daily feed intake, and the interaction effect of RPC×DE was highly significant between days 20 to 60 of the experiment. The overall average daily gain and feed to gain indices were not significantly affected by RPC supplementation at any energy level; however, RPC supplementation in a lowenergy diet tended to improve these indices. There were no significant effects of RPC×DE on slaughter performance, meat guality or serum biochemical indices; however, RPC supplementation resulted in lower drip loss, cook loss and shearing force, with a tendency to improve meat quality. Another positive effect of RPC supplementation was the enhancement of serum albumin and the reduction of serum urea nitrogen levels. So, it can be suggested that RPC has a greater effect on growth performance when supplemented in low-energy diets, and RPC addition can improve meat quality.

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Introduction

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Choline is a vitamin-like nutrient that functions as a biological methyl donor, in various reactions in the body thus playing a major role in energy metabolism. Although it can be synthesized by most animals, it is not produced in quantities sufficient to meet their requirements; as a result, external supplementation is often required. In ruminants, choline is rapidly degraded in the rumen (Sharma and Erdman, 1989); therefore, a rumen-protected form of choline has been developed for its safe delivery to the small intestine for absorption.

Rumen-protected choline (RPC) is commonly used in the diets of dairy cattle and dairy goats during the transition and early lactation periods, which are characterized by negative hormonal and energy balances, as it can prevent the development of fatty liver and improve milk yield and composition (Hartwell et al., 2000; Pinotti et al., 2008). However, the use of RPC in fattening goats has received little attention. In a recent study from Yangzhou University, China, it was shown that dietary supplementation with 0.25% RPC can promote weight gain in young lambs and improve meat quality (Li et al., 2015). In a subsequent study conducted in Egypt during summer it was found that RPC supplementation at 20 g/buck/day in growing male Zaraibi goats under heat stress yielded the best growth performance, feed conversion and blood metabolite levels, and economic efficiency (Habeeb et al., 2017). These studies demonstrate that choline supplementation in growing sheep can effectively improve production performance and meat quality.

In the review on the effects of betaine on energy utilization in growing pigs, Cromwell et al. (1999) noted a greater response to betaine addition in animals fed lower-energy diets. As betaine is a chemical precursor of choline and has similar biological activity as a methyl donor, we suspected that choline may have a similar effect on fattening goats. We hypothesised that supplementation of RPC during fattening period would preferentially benefit goats fed low-energy diet, and expected an interaction of diet energy by RPC supplementation. The lack of ruminant feed has recently emerged to a serious issue in China, and has led to the development of some unconventional feeds from agricultural processing by-products for utilization in goat fattening. Most of these feeds have a common feature, i.e., highfibre and low-energy values, which reduce the diet energy content. As a result, addition of oil or fat-rich feed is required to increase the dietary energy level; however, this is restricted by animal metabolic physiology, and a more appropriate approach would improve the energy efficiency. This issue could be resolved if choline could achieve a greater response when fed with a lower-energy diet, as more energy would then be utilized for animal growth. Furthermore, as consumers are more concerned with meat quality, researchers now focus on the effects of feed additives in improving meat quality.

Therefore, we designed an experiment to evaluate the effects of RPC supplementation on the performance, meat quality and serum biochemical parameters of fattening goats at two different digestible energy levels. An interaction between dietary energy intake and RPC supplementation was expected.

Material and methods

The use of animals and experimental procedures were approved by the Jiangsu Academy of Agricultural Sciences Animal Care and Use Committee (China).

Animals and experimental design

The experiment was conducted at an experimental goat farm, in Suqian city, Jiangsu Province, China, from August to October 2018. In total, 40 six-month-old Cashmere goats, with an initial live body weight of 24.30 ± 2.64 kg, were randomly assigned to one of the four treatments, with 10 individuals per treatment. Diets were prepared according to the standard feed for meat-producing sheep and goats (MOA, 2014; NY/T 816-2004). The experiments were performed in a 2×2 factorial design consisting of two levels of RPC (0 or 0.2%) and either a low (10.89 MJ/kg) or high (11.94 MJ/kg) digestible energy (DE) content (Table 1). The RPC supplementation level was determined based on published literature and previous research results. The RPC, containing 25% choline chloride, was obtained from Shanghai Mei Nong Biotechnology Co., Ltd (Shanghai, China).

Table 1. Four dietary treatments arranged in a 2×2 factorial design

RPC supplementation level, %	Dietary DE level, MJ/kg
0	11.94
	10.89
0.2	11.94
	10.89

RPC - rumen-protected choline; DE - digestible energy

Animals were fed maize-soybean meal-based diets, and the different DE levels were obtained by replacing maize and soybean meal with whole cottonseed (Table 2).

The goats were housed in individual pens and were fed twice a day, at 8:00 and 17:00. Fresh water was available *ad libitum*. The experiment lasted for 70 days, with the first 10 days for acclimation and next 60 days as the formal experimental period. All goats were weighed at the beginning of the experiment, and then every 20 days till the end of the experiment. The offered and residual feeds were recorded daily. The average daily feed intake (ADFI), average daily gain (ADG) and feed to gain ratio (F/G) were calculated to evaluate the production performance.

Blood samples

Blood samples were collected from the jugular vein at the end of the experiment. Samples were allowed to clot for 30 min and then centrifuged at 1125 g for 15 min to obtain the serum, which was stored at -20 °C for further analyses. The blood samples were analysed in the General Hospital of Nanjing Military Region (China), using Hita-chi 7020 blood biochemical analyser (Hitachi, Tokyo, Japan)

Ingredient, % of DM	High-energy diet	Low-energy diet	Nutrient composition	High-energy diet	Low-energy diet
Maize (ground)	26.00	16.50	DM	90.40	90.46
Whole cottonseed	10.00	0	DE, MJ/kg	11.94	10.89
Soybean meal	3.00	6.50	CP, % DM	14.03	14.02
Wheat bran	8.00	8.00	NDF, % DM	34.58	38.29
Soybean hull	6.00	6.00	ADF, % DM	22.70	24.71
Sunflower meal	4.00	4.00	Ash, % DM	9.06	9.61
Maize germ meal	5.00	12.00	Ca, % DM	0.92	0.92
Soybean straw	8.00	17.00	P, % DM	0.42	0.42
Peanut straw	20.00	20.00	Price, Yuan/kg	1.65	1.51
Alfalfa block	5.00	5.00			
Yeast culture	0.30	0.30			
Rice husk	0.97	0.93			
NaCl	0.60	0.60			
CaHPO₄	0.42	0.25			
Limestone	0.76	0.77			
Bentonite	0.70	0.90			
Calcium propionate	0.25	0.25			
NaHCO ₃	0.50	0.50			
Premix ^a	0.50	0.50			
Total	100.00	100.00			

Table 2. Ingredients and nutrient composition of the used diets, on a dry matter (DM) basis

^a provided per kg of diet, IU: vit. A 4 000, vit. D₃ 400, vit. E 20 000; mg: FeSO₄ 69.03, CuSO₄ 17.6, K₂SO₄ 31.70, ZnSO₄ 57.14, MnSO₄ 44.03, CoCl₂ 0.25, Na₂SeO₃ 8.95, monensin 6, NaHCO₃ 740.91; DE – digestible energy, CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre

to detect for total bilirubin (T-Bili), total protein (TP), albumin (ALB), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and blood urea nitrogen (BUN) contents.

Carcass characteristics and meat quality

At the end of the experiment, 12 randomlyselected goats (three per group) were slaughtered after a 24-h fasting period to determine the meat quality. The head, skin and visceral organs were removed immediately, and the live and carcass weights were recorded. The eye muscle area was calculated, and samples of *musculus longissimus dorsi* were collected from the region between the 12th and 13th vertebrae to evaluate the dripping loss, pH, meat colour (45 min and 24 h), cooking loss and shear force.

Statistical analysis

All data were analysed using the SPSS software (Version 12.0; IBM, Armonk, NY, USA). Data were expressed as mean and standard error of the mean (SEM) of the whole, the differences between groups were tested using one-way analysis of variance (ANOVA) with Duncan's multiple range test. Results with P < 0.05 were considered statistically significant. Two-way ANOVA with generalized linear models was used to evaluate the main effects of RPC (with

vs without), energy level (high vs low) and their interaction (RPC×DE). Results with P < 0.05 were considered significant, P < 0.01 were extremely significant, and $0.05 \le P < 0.10$ indicated a tendency to differ.

Results

Growth performance of fattening goats

The effects of RPC supplementation in different energy level diets on growth performance are shown in Table 3. Both DE and RPC significantly affected the overall ADFI of goats (P < 0.05). The addition of RPC significantly improved the feed intake, particularly when applied with the low-energy diet, during days 40-60 of the experiment (P < 0.05). In contrast, it had a negative effect on feed intake, when applied with the high-energy diet, during the 2nd and 3rd phases (days 20–60) of the experiment (P > 0.05). Regarding the ADG, the main effect of RPC supplementation became significant during days 20-40 (P < 0.05). The ADG was significantly increased, by 60.88%, in the group fed the low energy diet (P < 0.05) in comparison with an increase of only 5.71% in the high-energy diet group (P > 0.05) and a tendency of RPC×DE was observed (P = 0.06).

Indices	-	DE, MJ/kg				Main effects ¹ , <i>P</i> -values			
		11.87 RPC, %		<u>10.53</u> RPC, %		- SEM	DE	RPC	RPC×DE
	lime								
		0	0.2	0	0.2	-			
ADFI, g/day	0–20 days	1345.79ªb*	1432.63 ^{b*}	1250.00ª*	1255.25ª*	25.68	<0.01	0.36	0.41
	20–40 days	1618.50ªb^	1589.25ª^	1567.50ª^	1665.50 ^{▷^}	11.96	0.58	0.14	<0.01
	40–60 days	1692.50 ^{b#}	1644.00 ^{b^}	1490.00ª^	1718.25 ^{▷^}	16.4	<0.05	<0.01	<0.01
	Overall	1552.26⁵	1555.29 ^b	1435.83ª	1546.33 ^b	14.26	<0.05	< 0.05	0.06
ADG, g/day	0–20 days	185.00 [*]	167.75*^	175.56*	216.25*	11.32	0.40	0.61	0.22
	20–40 days	205.75*	217.50 ^{b*}	135.00ª*	217.19 ^{b*}	10.38	0.06	< 0.05	0.06
	40–60 days	182.75 [*]	153.50ªb^	150.56ªb*	132.19ª^	6.35	<0.05	< 0.05	0.65
	Overall	191.17⁵	179.58ab	153.70ª	188.54ªb	6.16	0.24	0.33	0.06
Feed:gain ratio	Overall	8.31	9.02	9.55	8.59	0.27	0.47	0.82	0.14

Table 3. Effects of rumen-protected choline (RPC) at different dietary energy levels on growth performance

DE – digestible energy; SEM – standard error of the mean; ¹*P*-values of main effect of DE = high energy vs low energy, *P*-values of main effect of RPC = –RPC vs + RPC, *P*-values of cross effect of RPC×DE interaction; ADFI – average daily feed intake; ADG – average daily gain; ^{ab} – means with different superscripts in the same row are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**#} – means with different signs within the same column are significantly different at P < 0.05; ^{**}

However, the main effect of DE became significant during days 40–60 (P < 0.05), while the main effect of RPC weakened, showing a tendency to decrease the ADG (P = 0.05). Furthermore, for the overall experimental period, the effect of neither DE nor RPC on ADG was significant (P > 0.05). Nevertheless, it was found that RPC supplementation with low-energy diet was more effective and enhanced the ADG by 22.67% in comparison with a reduction of 6.06% in the high-energy diet.

Differences within columns for each trait indicated ADFI of the four groups in the 2nd stage (days 20–40) were all significantly higher than in the 1st stage (days 0–20) (P < 0.05), the largest increase was in low-energy diet with RPC addition group (32.8%). During the 3rd stage (days 40–60), the ADFI was further significantly enhanced with the increase of dietary energy level (P < 0.05), but the remaining groups had no significant difference with 2nd stage. Values of ADG in both the high-energy without RPC group and low-energy without RPC group did not significantly differ in the three stages (P > 0.05), but after adding RPC, both the lowenergy group and the high-energy group showed a significant decrease in ADG in the 3rd stage (40 to 60 days) (P < 0.05). The overall F/G was not significantly affected by DE, RPC or their interaction (P > 0.05). However, the effect of RPC supplementation on F/G was consistent with that on ADG, i.e., a greater response was observed for RPC supplementation in low-energy diet, resulting in a 10.05% reduction in F/G as compared with low- and high-energy diets without RPC supplementation.

Slaughter performance of fattening goats

The dressing percentage and eye muscle area were not significantly affected by RPC supplementation, dietary energy level or their interaction (P > 0.05) (Table 4).

Meat quality of fattening goats

As shown in Table 5, dietary energy had a significant main effect on meat shearing force (P < 0.05), which increased in line with increasing dietary energy level without RPC supplementation (P < 0.05). Dietary energy enhancement also tended to increase meat cooking loss (0.05 < P < 0.10). Furthermore, RPC supplementation had a significant

Table 4. Effects of rumen-protected choline (RPC) at different dietary energy levels on slaughter performance

•		,			•			
Indices	DE, MJ/k	g				Main effects ¹ , <i>P</i> -values		
	11.87 RPC, %		10.53	10.53 RPC, %				
			RPC, %			DE	RPC	RPC×DE
	0	0.2	0	0.2				
Weight before slaughter, kg	38.88	37.37	36.90	37.04	0.65	0.44	0.64	0.57
Carcass weight, %	17.67	17.75	16.39	17.06	0.46	0.36	0.72	0.78
Dressing percentage, %	45.44	47.39	44.37	46.02	0.65	0.38	0.21	0.91
Eye muscle area, cm ²	15.67	16.63	15.81	15.96	0.30	0.39	0.13	0.50

DE – digestible energy; SEM – standard error of the mean; ¹*P*-values of main effect of DE = high energy vs low energy, *P*-values of main effect of RPC = ¬RPC vs + RPC, *P*-values of cross effect of RPC×DE interaction

	DE, MJ/kg					Main effects ¹ , <i>P</i> -values			
Indices	11.87	11.87 RPC, %		- 10.53 RPC, %					
	RPC, %					DE	RPC	RPC×DE	
	0	0.2	0	0.2					
Drip loss, %	4.28	3.98	4.35	4.23	0.18	0.71	0.61	0.83	
Cook loss, %	32.93 ^b	31.65⁵	32.63 ^b	28.07ª	0.72	0.08	<0.05	0.14	
Shear force, %	58.58ª	56.45 ^{ab}	55.51 ^b	55.15 ^b	0.51	<0.05	0.13	0.27	
Meat colour									
L _{45min}	36.52	38.15	37.50	36.97	0.92	0.96	0.80	0.62	
a _{45min}	17.44	16.71	18.66	15.46	0.72	0.99	0.22	0.43	
b _{45min}	5.46	5.59	6.32	5.24	0.29	0.70	0.46	0.36	
Meat colour									
L _{24h}	40.38	40.83	40.95	41.93	0.80	0.66	0.71	0.89	
a _{24h}	13.74	14.62	16.29	14.86	0.48	0.17	0.78	0.25	
b _{24h}	7.27	7.07	8.30	8.30	0.38	0.21	0.90	0.90	
pH _{45min}	6.27	6.40	6.18	6.11	0.06	0.17	0.82	0.41	
pH _{24h}	5.83	5.81	5.82	5.81	0.04	0.96	0.91	0.96	

Table 5. Effects of rumen-protected choline (RPC) at different dietary energy level on meat quality

DE – digestible energy; SEM – standard error of the mean; ¹*P*-values of main effect of DE = high energy vs low energy, *P*-values of main effect of RPC = –RPC vs + RPC, *P*-values of cross effect of RPC×DE interaction; L – brightness, a – redness, b – yellowness; ^{ab} – means with different superscripts in the same row are significantly different at P < 0.05

Table 6. Effects of rumen-protected choline (RPC) at different dietary energy levels on blood biochemical indices

	DE, MJ/kg					Main effects ¹ , <i>P</i> -values		
Indices	11.87 RPC, %		10.53			DE	RPC	RPC×DE
			RPC, %	RPC, %				
	0	0.2	0	0.2				
Total bilirubin, µmol/l	14.05	12.67	13.95	14.17	0.49	0.50	0.58	0.44
Total protein, g/l	69.05	65.76	69.86	69.29	0.69	0.11	0.15	0.31
Albumin, g/l	31.99 ^{ab}	35.50⁵	29.30ª	33.31 ^{ab}	0.77	0.08	<0.01	0.85
Glucose, mmol/l	2.77ª	3.09 ^b	3.05 ^{ab}	3 .11⁵	0.05	0.15	0.07	0.21
Aspartate aminotransferase, U/I	54.40	55.03	57.56	52.31	2.47	0.97	0.67	0.59
Alanine, U/I	54.62	57.67	63.83	59.27	1.99	0.19	0.85	0.35
Blood urea nitrogen, mmol/l	17.52⁵	15.64 ^{ab}	15.48 ^{ab}	13.78ª	0.42	<0.05	<0.05	0.90

DE – digestible energy; SEM – standard error of the mean; ¹*P*-values of main effect of DE = high energy vs low energy, *P*-values of main effect of RPC = –RPC vs + RPC, *P*-values of cross effect of RPC×DE interaction; ^{ab} – means with different superscripts in the same row are significantly different at P < 0.05

main effect on cooking loss (P < 0.05), which was decreased by 13.97% in the low-energy diet group (P < 0.05) in comparison with a decrease of only 3.89% in the high-energy diet group (P > 0.05). Meat colour and pH values measured 45 min and 24 h after slaughter were not affected by RPC or DE (P > 0.05), and no interaction effect of RPC×DE could be detected for any meat quality parameter tested (P > 0.05).

Blood biochemical parameters of fattening goats

Blood biochemical parameters are listed in Table 6. RPC exerted a significant effect on ALB (P < 0.05) and BUN (P < 0.05), and had a tendency to affect GLU (0.05 < P < 0.1). DE had a significant effect on BUN (P < 0.05), and a tendency to affect

ALB (0.05 < P < 0.1). However, their interaction did not have any significant effect (P > 0.05). ALB concentration was increased by RPC supplementation at both energy levels (P > 0.05). GLU concentration was significantly improved by RPC supplementation in high-energy diets (P < 0.05). BUN concentration decreased after RPC supplementation at both dietary energy levels (P > 0.05). No significant effect of RPC×DE interaction was detected for any blood biochemical parameter (P > 0.05).

Discussion

Effect on growth performance. In numerous studies it was reported an increase in dry matter intake (DMI) of dairy cows supplemented with RPC

(Grummer, 2012; Soltan et al., 2012), whereas in some studies no effect of RPC on the DMI of cows and goats was found (Bryant et al., 1999; Garg et al., 2012). The effect of choline on animal feed intake is dependent on many factors, such as its purity, the amount added, protection form and animal body condition. In the present study, DMI was significantly increased by RPC addition, which may be attributed to the improvement of body metabolism, resulting in higher feed intake. Supplementation of RPC increases milk production in cows, improves milk components and increases ADG in heifers, and these effects are independent of the prepartum caloric intake (Zenobi et al., 2018). However, in the present study, we observed a tendency of the RPC×DE interaction to affect goat growth performance. The effect of RPC was greater when supplemented in low-energy diets. A similar response was observed by Habeeb et al. (2017), who reported the best growth, feed conversion, blood metabolite levels and economic efficiency in Zaraibi goats fed diets supplemented with RPC, particularly subjected to heat stress during the Egyptian summer. We assumed that RPC supplementation may have a greater effect under stress conditions, such as high temperature or low dietary energy. In a review of the effects of betaine on energy utilization in growing pigs it was also documented that betaine can retain water inside the cell at lower-energy expenditure, thus reducing the energy requirements for cellular maintenance, allowing more energy to be used for animal growth (Lipiński et al., 2012). The addition of betaine can increase the energy availability, thus improving the efficiency of low-energy diets (Schrama et al., 2003). Betaine is a product of choline oxidation, and thus, these findings support our results; however, the specific mechanism requires further investigations.

Comprising the results of three stages of the study, it can be seen that the addition of RPC had a significant effect on the increase of ADFI especially in low-energy diets. The addition of RPC in low-energy diets could significantly increase the ADG during the first two stages of the experiment, but the effect of RPC was weakened in the last stage of the experiment. It is consistent with the main effect analysis result (presented in Table 3). The main effect of RPC on ADG was weakened at the last stage of the experiment. Therefore, RPC is more suitable to add early in the low-energy diets, and its mechanism needs further studies.

Effect on slaughter performance and meat quality. RPC addition did not have a significant influence on slaughter performance. However, it resulted in lower drip loss, cook loss and shearing force, thus exhibiting a tendency to improve meat quality. This is consistent with the findings of Li et al. (2015). Choline occurs in the cell membranes as lecithin, and improves the structural integrity of cell membranes, thereby enhancing meat quality. Choline can be also transformed into phosphatidylcholine, which is an important component of the cell membrane. The ratio of phosphatidylcholine to phosphatidylethanolamine is a key regulator of membrane integrity, and a decrease in this ratio can even lead to cell death (Yen et al., 1999). Thus, RPC supplementation may increase phosphatidylcholine synthesis, thereby increasing the ratio of phosphatidylcholine to phosphatidylethanolamine, resulting in improved cell integrity, and lower drip loss and shearing force of meat.

Effects on haematological parameters. ALB and TP indicate immunity functions in animals. Our results are consistent with a previous report of increased ALB percentage in RPC-supplemented cows (Rahmani et al., 2014). The increase in GLU following RPC supplementation may be attributed to choline-mediated increase in glucose transport to provide energy for peptide synthesis, as reported by Rahmani et al. (2014). In the present study, any effect of RPC supplementation on AST and ALT was found, indicating that the goats were in good health. This result was consistent with the findings of Habeeb et al. (2017), who did not observe any effect of RPC supplementation on plasma AST and ALT in growing male Zaraibi goats. Serum urea nitrogen is an important indicator of nitrogen metabolism in the body. Decreased serum urea nitrogen indicates negative changes in protein decomposition and an increase in protein precipitation. We observed a significant reduction of BUN following RPC addition, whereas an increase in the dietary energy level significantly increased the BUN concentration. This indicates that RPC supplementation can increase the utilization of protein in the feed, which cannot be achieved by an increase in the dietary energy level alone.

Conclusions

An improvement in the growth performance, especially in the early fattening period, of fattening goats fed a low-energy diet with rumen-protected choline (RPC) addition was observed. Consumption of such a diet resulted in lower drip loss, cook loss and shearing force of meat, and showed a tendency to improve meat quality. So, using RPC as a feed additive in fattening goat feeding is effective and has economic advantages. It was shown that lowenergy diet has a positive impact on goat production performance (close to high-energy diets productive results), and results in good meat quality.

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