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Research Article

Effect of Dietary Glycerol Addition on Growth Performance, Serum Biochemical Indexes, Carcass Traits, Fat Deposition, and Meat Quality in Fattening Period Kazakh Sheep

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Abstract

Glycerol has been evaluated as a safe and promising animal feed. It can replace traditional energy foods and reduce competition with major animal husbandry systems. In this study, 40 fattening Kazakh ewes (18 months of age) were selected to investigate the dynamic response of growth performance, serum biochemical indexes, fat deposition, carcass traits and meat quality to different levels of glycerol (0, 1%, 7%, and 12% DM). The results showed that glycerol could improve sheep growth performance, increase fat deposition, increase carcass dressing percentage and improve meat quality. Among them, the Gly7% group had significant positive effects (P<0.05) on FBW, ADG, GLU, TP, ALB, TG, HDL-C, fat deposition, HCW, CCW, HCD and EE; and significant negative effects (P<0.05) on FCR, ADFI, TC, LDL-C, a*, b* and SF. In conclusion, the recommended supplementation of glycerol in the diet of Kazakh ewes is 7% DM for optimal fattening performance without compromising health. This study may provide a theoretical basis for the rational utilization of food-grade glycerol in sheep diets.

Keywords: Kazakh sheep, Glycerol, Fat deposition, Growth performance, Carcass traits

Introduction

Kazakh sheep is a meat and fat dual-purpose rough wool sheep breed, with adaptability, cold resistance, rough feeding, stable genetic traits, disease resistance, and other excellent characteristics. They are quick to catch fat in summer and fall and have high meat and fat production performance [1]. Inadequate forage in winter and spring leads to insufficient nutritional intake and poor body condition of sheep, which seriously affects the production and reproductive performance of ewes in the coming year. Therefore, empty ewes need to supply energy and restore body condition in the spring. Crude glycerol is a by-product of the conversion of vegetable oils or animal fats into biodiesel, and its main component is glycerol [2]. Glycerol has an energy value similar to corn. It is a safe and promising animal feed that can enter the animal feed market as a safe dietary ingredient for animals, thus replacing traditional energy feeds [3,4].

In recent years, a large number of studies have explored the potential of adding glycerol to ruminant diets. Cheng and Huang [5] reported that glycerol can improve the economic efficiency of beef cattle rearing. Barros et al.[6] and Van Cleef et al.[7] found that the use of crude glycerol only reduced the nutrient intake of the animals and had no significant effect on growth performance. Orrico et al.[8] concluded that the addition of 7.5% crude glycerol to the diet of fattening lambs had a more positive effect in terms of reducing dry matter intake, improving feed conversion and reducing feeding costs. Ribeiro et al.[9] suggested that the addition of 70 g/kg of crude glycerol to lamb diets contributed more positively to increases in slaughter weight, carcass weight and improved carcass traits. However, Brant et al.[10] demonstrated that replacing energy diets with glycerol significantly reduced lamb final weight, average daily gain and cold carcass yield. The effect of glycerol addition to ruminant diets on the above

indicators varied from experiment to experiment, thus more in-depth studies are needed.

Current research on glycerol has focused on exploring its effects on feed intake, nutrient digestibility, rumen fermentation and sensory traits. However, the effect of food-grade glycerol as an energy supplement on fat deposition and fattening in Kazakh ewes has not been reported. Therefore, this study aimed to evaluate the effect of glycerol as an energy supplement in feed on growth performance, serum biochemical indexes, carcass traits, fat deposition and meat quality of fattening Kazakh sheep and to determine the appropriate dosage in diets. Ultimately, a theoretical basis was laid for the application of food-grade glycerol in sheep farming.

Material and Methods

Ethical Statement

This study was approved by the Bioethics Committee of Shihezi University (Approval no: A2021-38). All sheeps were kept experimentally and euthanized in strict accordance with the committee's guidelines. During the experiment, every effort was made to minimize suffering by the animals.

Animals, Experimental Design and Feed

Forty healthy Kazakh ewes (18 months of age; initial body weight = 32.55±3.13 kg) were selected in the spring and randomly divided into 4 groups of CON, Gly1%, Gly7% and Gly12% according to the level of glycerol supplementation, with 10 replicates in each group. All sheep were fed a basal diet for a 104-d feeding period (comprising a 14-d adaptation period and a 90-d experimental period). The diet was formulated according to The National Research Council (NRC) (2007) ewes nutritional standard, and its composition and nutritional levels are listed in *Table 1*. And the glycerol used in this experiment was food-grade glycerol (License No. SC20137148200057, purity: 99.88%, combustion energy: 4.32 Mcal/kg), which was supplied by Shandong Xuanyang Biotechnology Co. Ltd (Shandong, China).

Forty sheep were shorn and vaccinated before the experiment and weighed and housed in groups in a semi-open barn with a consistent feeding environment in terms of temperature, ventilation and light. Sheep were fed the basal diet twice a day (at 08:00 and 19:00) with ad libitum feeding and watering. Glycerol was dissolved in water and administered 1 h after morning feeding each day according to the glycerol concentration corresponding to the different groups. The amount of sheep fed to each group was adjusted to exceed the total feed intake of each group by about 3% on the previous day. At the end of the feeding experiment, six sheep from each group with final body weights close to the group average were selected and euthanized after 12 h of fasting and water deprivation.

Determination of Growth Performance

The body weight was recorded as initial body weight (IBW) on the 1st day and final body weight (FBW) on the 90th day of the experiment period and was weighed every 30d during the experiment period. The amount of feed and leftovers from each group were collected and weighed daily to calculate the average daily feed intake of sheep in the group (ADFI=(amount fed on the day-leftovers on day 2)/10). Finally feed conversion ratio (FCR=ADFI/ADG) was calculated.

Serum Collection and Analysis

Serum was prepared by collecting 5 mL of jugular vein blood before morning feeding on days 1, 30, 60, and 90

Table 1. *Ingredients and chemical compositions of basal diet (%, of DM)*

Ingredients	Content, %	Chemical Composition[2]	Content, %
Alfalfa hay	40.00	Net energy (Mcal/kg)	2.62
Alfalfa silage	35.00	Dry Matter (DM)	83.375
Oat grass	5.00	Crude protein (CP)	17.50
Soybean meal	7.90	Ash	8.63
Corn	8.50	Ether extract (EE)	2.90
Baking soda	1.50	Calcium	0.49
Premix[1]	1.00	Phosphorus	0.41
Sodium chloride	0.80	Neutral detergent fiber(NDF)	44.94
Lysine	0.30	Acid detergent fiber(ADF)	35.59
Total	100.00		

[1] *Provided the following per kilogram of diet: 3600 mg of Fe (as ferrous sulfate), 4000 mg of Zn (as zinc sulfate), 400 mg of Cu (as copper sulfate), 20 mg of Na (as sodium chloride), 2000 mg of Mn (as manganese), 40 mg of I (as potassium iodide), 20 mg of Co (as cobalt sulfate), 400,000 IU of vitamin A, 40,000 IU of vitamin D_3, and 8,000 IU of vitamin E.*
[2] *Energy level were predicted and the rest nutrient levels were measured*

of the positive test period, respectively. Total protein (TP), albumin (ALB), globulin (GLB), creatinine (Cre), blood urea nitrogen (BUN), glucose (GLU), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AKP) were measured using commercially available kits from the Institute for Construction Bioengineering (Nanjing, Jiangsu, China). Experiments were performed on a full-wavelength enzyme labeler-microplate spectrophotometer (Thermo Fisher Scientific, USA) instrument as described by Zheng et al.[11].

Determination of Fat Deposition and Carcass Traits

Live weight before slaughter (LWBS), organ weights, and fat weights of each site (pericardial fat, perirenal fat, and tail fat) were weighed and recorded, and then an index was calculated for each organ (organ index= (organ weight/LWBS) × 100). The carcasses were weighed as hot carcass weights (HCW) after standing for 30 min and cold carcass weights (CCW) after 24 h of acid drainage at 4°C, and the hot carcass dressing percentage (HCD = (HCW/LWBS) × 100) and cold carcass dressing percentage (CCD = (CCW/LWBS) × 100) were calculated. Loin eye muscle area (LEA = Length × width × 0.7) and back-fat thickness (BFT) were measured as described by Coria et al.[12] at the longissimus dorsi between the 12th and 13th ribs on the left side of the carcass.

Determination of Meat Quality

A sample of the longissimus dorsi of each sheep was taken and measured for meat color and pH. PH was determined at 45 min and 24 h after slaughter using a portable acidimeter. Meat color was recorded 45 min after slaughtering with a meat colorimeter (MATTHAUS, OPTP-STAR, Germany), including L* (brightness), a* (red) and b* (yellow). Meat color and pH were measured three times at different parts of the sample and the average of the three points was taken to determine the final values. Samples of the longissimus dorsi were taken for the determination of drip loss, cooking loss and shear force. Each sample was trimmed into a 2 cm square, weighed and recorded as W1, hung in a bag, stored at 4°C for 24 h and then blotted and weighed as W2, which was used to calculate the drip loss (DL = (W1-W2)/W1 × 100). About 50 g of each muscle sample was taken, weighed and recorded as w1, and heated in a sealed water bath at 80°C for 1 h. The meat samples were removed and cooled to room temperature in cold water, drained and weighed and recorded as w2, which was used to calculate the cooking loss (CKL = (w1-w2)/w1 × 100). The meat samples after cooking loss determination were cut into 1cm×1cm×3cm sizes along its muscle fiber direction for shear force (SF) determination. Each sample was randomly selected at three different locations and cut perpendicular to the muscle fiber direction using a digital tenderizer (Model: C-LM3B), and the average value was taken at the end.

The chemical composition of the longissimus dorsi and diets was determined according to the recommendations of AOAC (2008). Conventional nutrients were determined according to the following methods: dry matter (method 934.01); crude protein (CP) (N × 6.25; method 984.13); ether extract (EE) (method 920.39); crude ash (Ash) (method 942.05) were determined [13].

Statistical Analysis

The experimental data were analyzed using SPSS 26.0 software. Data on ADG, ADFI, FCR and serum biochemical indexes were analyzed using a linear mixed-effects model. The method used comprised treatment, period, and treatment × period as fixed factors, and sheep as a random factor to assess the effect of different glycerol levels of repeated measures over time on the same sheep. Means were estimated using least-square means, with statistical significance between groups determined using the Duncan test [14,15]. The remaining data were analyzed by one-way ANOVA and Duncan test. The results were expressed as the mean ± SEM. A statistical significance was considered as $P<0.05$, and a trend was defined as $0.05\leq P<0.10$.

Results

As can be seen in *Table 2*, the difference in IBW between the groups was not significant ($P>0.05$), and FBW was significantly increased in the Gly7% group ($P<0.05$). Period and treatment × period had no significant effect on ADG and FCR ($P>0.05$), while Period had a significant difference on ADFI ($P<0.05$), especially, ADFI was significantly reduced on the 60th and 90th d. The differences in ADG, ADFI and FCR between the groups were significant ($P<0.05$), with the Gly 7% group significantly increasing ADG by about 28.40% and decreasing ADFI and FCR by about 1.63% and 35.57%, respectively, compared with the CON group.

The blood biochemical indexes of Kazakh sheep are shown in *Table 3*. Treatment × period had a significant effect on serum GLU, TG, HDL-C and LDL-C levels ($P<0.05$), but not on other indexes ($P>0.05$). In terms of treatment, serum concentrations of GLU, TP, ALB, TG, HDL-C and TG were significantly higher and serum concentrations of TC and LDL-C were significantly lower in the Gly7% group compared to the CON group. However, other biochemical indexes were not significantly different between the groups ($P>0.05$). The effects of the experiment period on serum GLU, LDL-C, TG, TC and AKP parameters were all significantly different ($P<0.05$). Among them, serum

Table 2. *Effect of glycerol supplementation in diets on growth performance of Kazakh sheep (n=10)*

Item	Experimental Diets				SEM	Period			SEM	P-Value		
	CON	Gly1%	Gly7%	Gly12%		30d	60d	90d		Treatment	Period	Treatment × Period
IBW/kg	32.58	32.52	32.54	32.56	0.450	-	-	-	-	1.000	-	-
FBW/kg	45.30^{b}	46.66^{ab}	48.60^{a}	45.02^{b}	0.501	-	-	-	-	0.039	-	-
ADG/(g/d)	142.78^{b}	155.56^{ab}	183.33^{a}	131.67^{b}	6.640	153.00	157.83	150.67	6.606	0.018	0.891	0.472
ADFI/(g/d)	1.23^{a}	1.22^{ab}	1.21^{b}	1.22^{ab}	0.003	1.29^{a}	1.19^{b}	1.18^{b}	0.001	0.004	<0.001	0.154
FCR	11.16^{a}	8.56^{bc}	7.19^{c}	10.33^{ab}	0.399	9.09	9.18	9.67	0.397	0.001	0.780	0.565

^{a,b} *Values within a row with different superscripts differ significantly at P<0.05; CON, Gly 1%, Gly 7% and Gly 12% group was fed the basal diet supplemented with glycerol at 0%, 1%, 7%, and 12% (DM basis) per sheep*

Table 3. *Effect of glycerol supplementation in diets on blood biochemical indexes of Kazakh sheep (n=10)*

Item	Experimental Diets				SEM	Period				SEM	P-Value		
	CON	Gly1%	Gly7%	Gly12%		0d	30d	60d	90d		Treatment	Period	Treatment × Period
GLU (mmol/L)	2.37^{b}	2.54^{ab}	2.60^{a}	2.48^{ab}	0.034	2.24^{c}	2.48^{b}	2.73^{a}	2.53^{b}	0.031	0.040	<0.001	0.016
Cre (μmol/L)	49.11	55.71	59.25	52.53	1.583	49.65	59.06	55.25	52.65	1.554	0.100	0.128	0.713
TP (g/L)	55.29^{b}	55.64^{b}	56.44^{a}	55.39^{b}	0.137	55.52	55.98	55.81	55.44	0.116	0.011	0.400	0.985
ALB (g/L)	21.61^{b}	22.26^{ab}	22.63^{a}	22.58^{a}	0.132	21.96	22.03	22.44	22.64	0.130	0.015	0.152	0.468
GLB (g/L)	33.68	33.38	33.81	32.81	0.179	33.56	33.96	33.37	32.80	0.175	0.173	0.118	0.896
BUN (mmol/L)	5.15	5.13	5.13	5.19	0.010	5.13	5.13	5.16	5.18	0.010	0.080	0.107	0.249
TG (mmol/L)	0.55^{b}	0.57^{ab}	0.60^{a}	0.54^{b}	0.008	0.55^{ab}	0.53^{b}	0.60^{a}	0.58^{ab}	0.008	0.014	0.013	0.030
HDL-C (mmol/L)	0.97^{b}	0.99^{ab}	1.02^{a}	0.95^{b}	0.010	0.96	0.97	1.02	0.98	0.010	0.023	0.090	0.030
LDL-C (mmol/L)	0.61^{a}	0.56^{ab}	0.54^{b}	0.61^{a}	0.012	0.65^{a}	0.61^{a}	0.52^{b}	0.53^{b}	0.011	0.039	<0.001	0.034
TC (mmol/L)	1.58^{a}	1.51^{ab}	1.34^{b}	1.31^{b}	0.037	1.28^{b}	1.45^{ab}	1.54^{a}	1.48^{ab}	0.036	0.014	0.048	0.954
ALT (U/L)	22.46	21.63	21.49	21.91	0.240	22.60	22.19	21.07	21.64	0.235	0.466	0.105	0.981
AST (U/L)	89.25	89.12	89.88	88.67	0.472	89.92	89.52	89.53	87.95	0.441	0.748	0.185	0.492
AKP (U/L)	86.92	85.63	85.08	87.24	0.419	87.65^{a}	86.82^{ab}	85.07^{b}	85.33^{b}	0.410	0.161	0.012	0.774

^{a,b,c} *Values within a row with different superscripts differ significantly at P<0.05; CON, Gly 1%, Gly 7%, and Gly 12% group was fed the basal diet supplemented with glycerol at 0%, 1%, 7% and 12% (DM basis) per sheep*

Table 4. *Effect of glycerol supplementation in diets on fat deposition of Kazakh sheep (n=6)*

Item	Experimental Diets				SEM	P-Value
	CON	Gly1%	Gly7%	Gly12%		
Pericardial fat	67.42	73.24	75.35	68.88	1.885	0.433
Perirenal fat	96.70^{b}	119.62^{a}	124.55^{a}	98.19^{b}	4.044	0.012
Tail fat	939.17^{b}	976.55^{ab}	1048.10^{a}	923.95^{b}	17.126	0.036
Total fat	1003.29^{b}	1169.40^{ab}	1248.00^{a}	1091.02^{b}	19.427	0.007

^{a,b} *Values within a row with different superscripts differ significantly at P<0.05; CON, Gly 1%, Gly 7%, and Gly12% group was fed the basal diet supplemented with glycerol at 0%, 1%, 7% and 12% (DM basic) per sheep*

GLU, TG, and TC levels were significantly higher, and LDL-C and AKP levels were significantly lower on 60 d. However, HDL-C content tended to increase (P=0.090) on 60 d (1.02 mmol/L vs. 0.96 mmol/L).

As shown in *Table 4*, perirenal fat, tail fat and total fat weights differed significantly (P<0.05) among the groups. Compared to the CON group, perirenal fat was significantly increased by about 23.70% and 28.80% in the Gly 1% and Gly 7% groups, and tail fat and total fat were significantly increased by about 11.60% and 24.39% in the Gly 7% group. Fat weight in the CON group was lower than in each glycerol group, except in the tail fat, where fat weight was slightly higher than in the Gly 12% group. The

Table 5. *Effect of glycerol supplementation in diets on carcass traits of Kazakh sheep (n=6)*

Item	Experimental Diets				SEM	P-Value
	CON	Gly1%	Gly7%	Gly12%		
LWBS (kg)	45.40	46.53	48.50	44.90	0.537	0.073
HCW (kg)	19.89^{b}	20.61^{b}	22.18^{a}	19.83^{b}	0.323	0.022
HCD (%)	43.79^{b}	44.28^{b}	45.71^{a}	44.17^{b}	0.260	0.035
CCW (kg)	18.70^{b}	19.67^{ab}	21.00^{a}	18.77^{b}	0.332	0.037
CCD (%)	41.19	42.22	43.35	41.83	0.477	0.465
BFT (mm)	4.88	5.27	5.65	5.15	0.112	0.103
LEA (cm^2)	14.24	13.78	13.42	13.93	0.150	0.296

^{a,b} *Values within a row with different superscripts differ significantly at P<0.05; CON, Gly 1%, Gly 7%, and Gly 12% group was fed the basal diet supplemented with glycerol at 0%, 1%, 7% and 12% (DM basis) per sheep*

Table 6. *Effect of glycerol supplementation in diets on organ weights and indexes of Kazakh sheep (n=6)*

Organ	Item	Experimental Diets				SEM	P-Value
		CON	Gly1%	Gly7%	Gly12%		
Heart	Weight (g)	260.97	256.53	272.33	258.50	2.492	0.101
	PLWBS (%)	0.58	0.55	0.56	0.58	0.010	0.463
Liver	Weight (g)	503.27	570.15	631.87	563.95	18.826	0.111
	PLWBS (%)	1.12	1.30	1.33	1.09	0.050	0.230
Spleen	Weight (g)	75.27	78.07	77.20	72.70	1.111	0.346
	PLWBS (%)	1.17	1.17	1.16	1.16	0.003	0.890
Lungs	Weight (g)	467.40	470.57	480.60	463.37	4.481	0.596
	PLWBS (%)	1.03	1.01	0.99	1.04	0.013	0.704
Kidneys	Weight (g)	170.50	176.77	180.90	171.53	2.524	0.455
	PLWBS (%)	0.38	0.38	0.37	0.38	0.006	0.946

CON, Gly 1%, Gly 7% and Gly 12% group was fed the basal diet supplemented with glycerol at 0%, 1%, 7%, and 12% (DM basic) per sheep

Table 7. *Effect of glycerol supplementation in diets on meat quality of Kazakh sheep (n=6)*

Item	Experimental Diets				SEM	P-Value
	CON	Gly1%	Gly7%	Gly12%		
pH_{45min}	6.19	6.24	6.33	6.24	0.035	0.592
pH_{24h}	5.73	5.47	5.61	5.69	0.049	0.240
L*	34.13	38.60	36.57	36.00	0.658	0.111
a*	13.43^{a}	12.32^{b}	12.31^{b}	12.08^{b}	0.180	0.023
b*	9.83^{a}	9.59^{ab}	9.29^{b}	9.15^{b}	0.088	0.016
SF (N)	32.97^{a}	30.03^{ab}	28.13^{b}	30.67^{ab}	0.603	0.029
DL (%)	2.51	2.29	2.09	2.35	0.063	0.118
CKL (%)	30.67	29.45	28.35	29.80	0.328	0.082

^{a,b} *Values within a row with different superscripts differ significantly at P<0.05; CON, Gly 1%, Gly 7%, and Gly12% group was fed the basal diet supplemented with glycerol at 0%, 1%, 7% and 12% (DM basic) per sheep*

above results indicate that glycerol supplementation in the diet had a promotional effect on fat deposition, which was more pronounced in the Gly 7% group.

Carcass traits are shown in *Table 5*. Significant differences were observed in HCW, HCD and CCW between the groups (P<0.05). HCD increased in all glycerol groups compared to the CON group and increased with increasing glycerol levels in the Gly1% and Gly7% groups. However, there was a tendency for the Gly1% and Gly7% treatment groups to have higher LWBS (P=0.073) than the CON group (46.53 and 48.50 vs. 45.40 kg). In terms of organ weights and organ indexes *(Table 6)*, there were no

Table 8. *Effect of glycerol supplementation in diets on the chemical composition of the longissimus dorsi of Kazakh sheep (n=6)*

Item	Experimental Diets				SEM	P-Value
	CON	Gly1%	Gly7%	Gly12%		
Moisture (%)	68.61	70.29	71.01	69.73	0.372	0.129
CP (%)	23.27	23.63	24.64	23.17	0.229	0.080
EE (%)	5.05^{b}	5.22^{ab}	5.40^{a}	5.20^{ab}	0.043	0.025
Ash (%)	1.28	1.24	1.24	1.25	0.006	0.176

^{a,b} *Values within a row with different superscripts differ significantly at P<0.05; CON, Gly 1%, Gly 7% and Gly12% group was fed the basal diet supplemented with glycerol at 0%, 1%, 7% and 12% (DM basic) per sheep*

significant differences (P>0.05) between the groups. Liver and kidneys weights were increased in all glycerol groups as compared to the CON group, with the Gly7% group maximizing the increase by about 25.55% and 6.10%. All glycerol groups decreased heart and lungs indexes and increased liver index.

The meat quality is shown in *Table 7*. The pH value of the slaughtered muscle was less than 7.0, which was weakly acidic, and the pH value decreased with the cooling time of the muscle, but the difference between the groups was not significant (P>0.05). The a*, b* and SF decreased significantly with increasing glycerol levels (P<0.05). There was a trend of decreasing CKL in the treatment group compared to the CON group (29.45%, 28.35% and 29.80% vs. 30.67%) (P=0.082). In terms of muscle chemical composition *(Table 8)*, after glycerol supplementation, the EE content of muscle was increased in all groups, including a significant increase of 6.93% in the Gly7% groups (P<0.05). In addition, the content of CP in the Gly1% and Gly7% groups tended to increase compared with the CON group (23.63% and 24.64% versus 23.27%) (P=0.080). However, there was no significant difference in moisture and Ash content between the groups (P>0.05).

Discussion

Piao et al.[16] found that replacing a portion of the molasses in the diet with 3.17% glycerol increased the intake of their concentrates, and hypothesized that glycerol was more effective than molasses in improving appetite. Studies have shown that adding 5% and 10% glycerol to diets can increase ADG, decrease FCR and limit feed intake [16-19]. Our experimental results were similar in that the increase in glycerol supplementation in the diet was accompanied by a significant limitation of ADFI, which gradually decreased over time, reaching a minimum on the 90 d of the experiment. We speculate that this may be because absorbed glycerol is metabolized in the liver to 3-phosphoglycerol, which then participates in the gluconeogenesis or glycolysis pathway, increasing the number of hepatic tricarboxylic acid cycles, which in turn stimulates satiety in sheep, leading to a decrease in feed intake. And Wang et al.[18] reported that ADG increases with increasing dietary energy levels. However, we found that the body weight and ADG of the highest energy Gly12% group did not follow this pattern. We considered that the excessive nutrient levels negatively affected the digestion and metabolism of the sheep, thus affecting their growth performance. Meanwhile, ADG was highest on d 60, suggesting that this is when sheep are physiologically at their best. A similar report is: Ding et al.[20] found that ADG and FCR were highest on d 45 in a 60-d experiment.

Serum biochemical indexes can reflect the body's absorption and metabolism of nutrients [21]. GLU levels are positively correlated with dietary energy levels, with low GLU levels indicating a low level of dietary nutrition or a reduced ability of the body to digest, absorb and utilize sugars. GLU content is influenced by dietary energy levels, with low GLU indicating a low level of dietary nutrition or a reduced ability of the body to digest, absorb and utilize sugars [22]. In the present study, the CON and Gly12% groups had lower GLU levels, which was consistent with their lower body weights and ADGs, suggesting that these groups had inadequate energy intake or lower utilization of sugars. Melanson et al.[23] demonstrated for the first time that starvation is concomitant with a decrease in blood glucose, which in turn regulates animal feeding, and the relationship between the level of ADFI and GLU concentration in the present experiment is consistent with the above relationship. In the present study, we found that TG levels were significantly higher in the Gly7% group, suggesting that dietary supplementation with 7% glycerol promotes fat synthesis, according to the report of Cheng et al. [24]. However, elevated TG leads to associated metabolic disturbances such as decreased HDL-C and increased LDL-C [25]. We found that as TG increased, HDL-C instead increased and LDL-C instead decreased, thus inferring that glycerol supplementation does not lead to metabolic disorders in animals. Serum TP and ALB reflect the ability of the liver to synthesize proteins. The TP and ALB levels increased after glycerol supplementation, and the TP levels were all lower than 60 g/L and the ALB levels were all higher than 20 g/L, which were in line with the healthy range reported by Braun et al.[26]. This suggests that glycerol supplementation does not affect the health of sheep and is beneficial in improving protein synthesis.

The nutritional level of the diet is a key factor influencing fat deposition in animals [27]. Glycerol had a significant effect on perirenal fat, caudal fat and caudal fat deposition in this study. Song et al.[28] found that tail fat and perirenal fat decreased the most when diets were energy restricted. Differently, we found that tail fat and perirenal fat in the CON group were significantly lower than those in the Gly1% and Gly7% groups, but the difference with the Gly12% group was not significant. This suggests that glycerol has a certain promotion effect on fat deposition in sheep, and the fat deposition was optimal when the supplementation amount was 7%. The pattern of change in fat deposition was similar to that of ADG and TG, which was also in line with our speculation above the energy provided by the Gly12% group exceeded the demand of sheep, and their growth and metabolism were inhibited in all aspects.

Diet energy levels are closely related to carcass traits [29]. We found that HCW, CCW and HCD were significantly higher in the Gly1% and Gly7% groups than in the CON group. This suggests that the supplementation of glycerol at appropriate levels in the diet has a positive effect on increasing the carcass dressing percentage of sheep. However, Chanjula et al.[30] found that the addition of 5%, 10% and 20% glycerol to the diet did not significantly affect the above indexes. This may be because the food-grade glycerol added in this experiment had a higher glycerol concentration than crude glycerol. In addition, LEA was not significantly different between groups, but LEA was lower in both glycerol groups than in the CON group, which is not by the idea that the greater the carcass weight, the greater the area of the loin and eye muscles [31]. It is hypothesized that the possible reason for this is the positive effect of glycerol on the deposition of fat and muscle in animals. The weight of visceral organs increased significantly with increasing nutritional levels [32,33]. However, in the present study, glycerol was found to have no significant effect and only increased the weight of the liver and kidneys. This may be related to the age of the animals; the organs of the adult ewes in the present study were fully developed and glycerol supplementation could only promote further development of organs involved in energy balance.

The pH is an important indicator of muscle glycolysis rate and the increase in lactate content after slaughter leads to a decrease in pH values to 5.3-5.8 [34]. The results of the present study were similar with pH values within the reported normal range [22]. The a* and b* of muscle decreased significantly with an increase in glycerol, suggesting a possible negative correlation between glycerol levels and flesh color. The L* of the muscle decreases with increasing energy [18], but this is not the case in our results, which may be related to the pH of the muscle, where L* increases as the final pH decreases [35]. In addition, we found that the muscles in each glycerol group had lower SF and higher EE content, with the Gly7% group having the highest EE content and the lowest SF. Our results are in line with the conclusion of Bezerra et al.[31] that there is a negative correlation between SF and EE. This suggests that glycerol promotes intramuscular fat deposition, which in turn improves muscle tenderness.

In conclusion, it was determined that glycerol supplementation was effective in increasing FBW, ADG, HCW, CCW, HCD and serum concentrations of glycolipid metabolites, and was effective in decreasing FCR and ADFI in Kazakh sheep. In addition, glycerol supplementation promotes fat deposition, increases intramuscular fat content, reduces SF and improves meat quality. Therefore, it is feasible to supplement glycerol in the diet of empty ewes for fattening to improve body condition and provide energy for reproductive performance. We suggest that optimal fattening can be achieved with a 7% glycerol supplementation in the diet of empty ewes.

Declarations

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author (Z. Zhao). The data are not publicly available due to privacy or ethical restrictions.

Ethical Statement: This study was approved by the Bioethics Committee of Shihezi University (Approval no: A2021-38). All sheeps were kept experimentally and euthanized in strict accordance with the committee's guidelines. During the experiment, every effort was made to minimize suffering by the animals.

Conflict of Interest: The authors have declared that no competing interests exist.

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