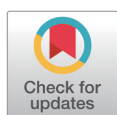


## Growth performance and blood profiles of Hanwoo steers at fattening stage fed Korean rice wine residue

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### Abstract

The aim of this study was to investigate the effects of Korean rice wine residue (RWR) on the growth performance and blood profiles of Hanwoo steers in the fattening stage. *In situ* and *in vivo* experiments were conducted to analyze rumen fermentation characteristics and total tract digestibility, respectively. Three cannulated Hanwoo steers (mean body weight: 448 ± 30 kg) were used in both analyses. The growth performance of 27 experimental animals in the fattening stage (initial body weight: 353.58 ± 9.76 kg) was evaluated after 13 months of feeding. The animals were divided into three treatment groups (n = 9/group). The treatments comprised total mixed ration (TMR) only (CON), TMR + 10% RWR (10% RWR), and TMR + 15% RWR (15% RWR). The diets of equal proportions were fed daily at 08:00 and 18:00 h based on 2% of the body weight. The animals had free access to water and trace mineral salts throughout the experiment. Supplementation of 15% RWR significantly decreased ( $p < 0.05$ ) the rumen fluid pH compared with the control treatment, but there was no significant difference in the total volatile fatty acid concentration. It also significantly increased ( $p < 0.05$ ) dry matter digestibility compared with the other treatments. The total weight gain and average daily gain of the animals in the RWR-supplemented groups were significantly higher ( $p < 0.05$ ) than those in the control group. Furthermore, the feed intake and feed efficiency of the RWR-supplemented groups were higher than those of the control group. Supplementation of RWR did not affect the alcohol, albumin, glucose, total cholesterol, triglyceride, and low-density lipoprotein concentrations, and aspartate aminotransferase and alanine transaminase activities in the blood; these parameters were within the normal range. The high-density lipoprotein and creatinine concentrations were significantly higher in the 15% RWR group, whereas the blood urea nitrogen concentration was significantly higher in the 10% RWR group than in the other groups. These results suggest that TMR with 15% RWR can serve as an alternate feed resource for ruminants.

**Keywords:** *In situ*, Hanwoo steer, *In vivo*, Korean rice wine residue

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#### Competing interests

No potential conflict of interest relevant to this article was reported.

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#### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

#### Authors' contributions

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Writing - review & editing: Ramos SC, Jeong CD, Lee SS.

#### Ethics approval and consent to participate

Animals used in this experiment and all experimental protocols were reviewed and approved by the Suncheon National University Animal Research Ethics Committee (SCNU IACUC-2018-01).

## INTRODUCTION

The supply of animal feed is being progressively reduced owing to an increase in human population and various natural phenomena, resulting in a high feed cost. Alternative feedstuffs composed of secondary products obtained during primary commodity processing have been identified [1]. By-product feeds are secondary products of primary product harvesting or processing. The by-products are of nutritional value to humans and animals, and hundreds of by-products are currently used in livestock feed. These by-products are of low cost, which is the principal variable of livestock production. It has been reported that food by-products may have a potential as animal feed because they are rich in crude proteins, cellulose, and water-soluble carbohydrates [2]. Therefore, the main strategies for successful livestock production are reducing feed cost and maintaining productivity. The total mixed ration (TMR) is commonly used in dairy farms and feedlots, as producers aim to reduce cost and increase economic scales [3]. Consequently, by-product feeds are valuable additions to the TMR system [4].

Wet distiller's by-products (WDB) such as Korean rice wine residues (RWR) can be a protein and energy source in cattle diets [5]. The high energy value of WDB can be attributed to the following reasons: it has three times higher fat content than the usual corn [6]; it enables rapid absorption of ethanol from the rumen, metabolization to acetate, and further utilization for energy or lipogenesis [7,8]; and cattle fed WDB consumed less starch and more corn fiber [9]. Moreover, WDB contains live and dead yeast cells, which show variable effects on ruminal fermentation; it also increases cellulolytic bacterial concentration in the rumen [9,10]. Wet distillers' grain (WDG) is more economic than dried distillers' grains with solubles (DDGS) and dried distillers' grains (DDG) owing to its ability to supply energy to ruminants and cost efficiency. The WDG lacks a drying process, and therefore, the price of WDG in South Korea (Korean won [KRW] 25 /kg) is lower than that of DDG (KRW 280 /kg) and DDGS (KRW 270 /kg) [5]. Moreover, supplementing 10%–15% RWR could decrease the feed cost to 8%–15% compared with non-supplementation [11]. However, the use of wet by-products is limited by high transportation cost owing to the high moisture content (60%–70%). The shelf-life of wet by-products is typically 3–7 days, but it is related to environmental temperature. Moreover, a sizeable operation is necessary to use a semi-load quantity before the spoilage of wet by-products [12]. Furthermore, the agricultural sector lacks sufficient data on the characteristics and effects of WDG. Kim et al. [5] did not observe any negative effects of up to 10% WDG in the TMR on Hanwoo steer performance. Jeong et al. [11] identified 681 Korean traditional rice wine-processing companies in Korea, producing approximately 83,808 tons of RWR. Rice wine-manufacturing companies usually dispose RWR in landfills or by burning, and thereby causing environmental problems. Thus, strategies to efficiently utilize industrial by-products are necessary. The aim of this study was to evaluate the effects of rice wine residue as a feed substitute on the growth performance of Hanwoo steers in the fattening stage.

## MATERIALS AND METHODS

### Animal care

This study was conducted at the Suncheon National University (SCNU) animal farm and in the Ruminant Nutrition and Anaerobe Laboratory, Department of Animal Science and Technology, SCNU, Jeonnam, South Korea. The animals used in this experiment and all of the experimental protocols were reviewed and approved by the Suncheon National University Animal Research Ethics Committee (SCNU IACUC-2018-01).

### Rumen fermentation characteristic (*in situ*) and total tract digestibility analyses (*in vivo*)

Three cannulated Hanwoo steers (mean body weight:  $448 \pm 30$  kg) were used in this study. The animals were individually housed in pens and distributed into a  $3 \times 3$  Latin square design. Each period lasted for 24 days (d), with 20 d adaptation and 4 d for the experiment and sampling. The experimental diets (TMR only [CON]; TMR + 10% rice wine residue/dry matter [DM] [10% RWR]; TMR + 15% RWR [15% RWR]) were fed daily in equal proportions at 08:00 and 18:00 based on 2% of the body weight. All of the animals had free access to fresh drinking water and trace mineral salts throughout the experiment. Ruminant fluid was collected from the rumen 6 h after feeding on day 24 of each period. The rumen fluid pH and volatile fatty acids (VFA) were measured via a pH meter (Pinnacle pH meter M540, Corning, NY, USA) and gas chromatography (GC; Varian CP-3800), respectively. Feces were collected daily and immediately stored at  $-20^{\circ}\text{C}$ . Rumen DM disappearance of samples was determined according to the protocol of Van Emon et al. [13] with minor modifications. Each samples were dried in an oven at  $80^{\circ}\text{C}$  for 48 h and ground to pass through a 1 mm screen. Ground samples were placed in a nylon bag ( $5 \times 10$  cm,  $50 \pm 10$ -micron porosity; R510, Ankom Technology, Macedon, NY, USA), and then suspended in the rumen of three cannulated Hanwoo steers. Nylon bags were removed from the rumen after incubation and rinsed in running tap water prior to the next procedure. Bags were again dried in an oven under the same condition as above and residual material was weighed for the determination of rumen DM disappearance [13]. All of the samples were analyzed for total nitrogen, DM, organic matter [14], neutral detergent fiber, and acid detergent fiber [15].

### Growth performance and blood profiling of Hanwoo steers in the fattening stage

The Korean rice wine residue (RWR) contains 49% DM, 9.18% crude protein, 0.66% ether extract (EE), 0.88% crude fiber, 0.35% ash, 37.07% nitrogen-free extract, and 5% ethanol. The RWR was stabilized and drained for 48 h before mixing into the animal feed. The TMR was based on the standard feed composition provided by the Korean National Institute of Animal Science (2007). The chemical composition measurements were based on the guidelines of the Association of Official Analytical Chemists [14]. Twenty-seven, Hanwoo steers (initial body weight:  $353.58 \pm 9.76$  kg) were used for 13 months feeding trial. The animals were divided into three treatment groups (nine animals per group) of three experimental diets - TMR only (CON), TMR + 10% RWR (10% RWR), and TMR + 15% RWR (15% RWR). The composition and nutrient content of the TMR containing different RWR inclusion rates (g/kg; DM basis) are shown in Table 1. The steers were housed in steel-constructed pens ( $5 \text{ m} \times 10 \text{ m}$ ) with sawdust bedding (three steers per pen) and were marked with numbered ear tags. The TMR diets were fed daily in equal proportions at 08:00 and 18:00 based on 2% of the body weight. The animals also had free access to fresh drinking water and trace mineral salts throughout the experiment. Blood samples were collected at the end of the feeding trial. The *in vivo* data included the feed intake, weight gain, and average daily gain. Feed efficiency was calculated as the ratio of weight gain to the amount of feed consumed. Five milliliters of blood were taken from the jugular vein of each animal into sterilized vacuum tubes (Green Cross MS, Korea) containing K3-EDTA at the end of the experimental period. The blood samples were centrifuged for 15 min at  $890 \text{ g}$  at  $4^{\circ}\text{C}$ , and the serum was stored at  $-20^{\circ}\text{C}$  until analysis. The contents of total protein (TP), total alcohol, blood urea nitrogen (BUN), albumin, glucose, and creatinine were analyzed using an automated blood serum biochemical analyzer (T Express Plus, USA). The serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride concentrations were analyzed by Green Cross (Yongin, Korea).

**Table 1.** Composition and nutrient content of the total mixed ration containing Korean rice wine residue at different proportions for Hanwoo steers in the fattening stage

Parameter	Treatment <sup>1)</sup>		
	CON	10% RWR	15% RWR
Feed composition (% of DM)			
Oat hay	18.87	18.40	19.75
Timothy hay	5.60	4.91	4.83
Alfalfa hay	5.62	4.93	4.85
Rice straw	4.78	4.47	4.58
Wheat grain	10.09	9.18	8.12
Corn	19.38	17.87	17.58
Corn gluten feed	20.73	19.12	15.99
Lupine	13.38	9.55	6.61
Salt	0.15	0.14	0.14
Limestone	0.15	0.15	0.14
RWR	0.00	10.07	15.04
Molasses	0.65	0.63	1.77
Vitamin-mineral mix <sup>2)</sup>	0.60	0.58	0.58
Nutrient content (% of DM)			
Crude protein	15.20	15.50	15.50
Ether extract	3.70	5.00	5.50
Ash	10.40	9.65	9.90
Crude fiber	18.00	15.90	15.40
Nitrogen free extract	52.70	53.95	53.70
Neutral detergent fiber	42.00	41.70	41.30
Acid detergent fiber	19.54	20.50	19.23
Total digestible nutrients	76.17	75.54	76.37

<sup>1)</sup>TMR only (CON); TMR + 10% RWR (10% RWR); TMR + 15% RWR (15% RWR).

<sup>2)</sup>Vitamin-mineral mix contained vit. A 2,650,000 IU, vit. D<sub>3</sub> 530,000 IU, vit. E 1,050 IU, niacin 10,000 mg, Mn 4,400 mg, Zn 4,400 mg, Fe 13,200 mg, Cu 2,200 mg, iodine 440 mg, and Co, 440 mg/kg of Grobic-DC provided from Bayer Health Care (Leverkusen, Germany).

RWR, Korean rice wine residue; DM, dry matter; TMR, total mixed ration.

### Statistical analysis

The data were analyzed using the one-way analysis of variance (ANOVA) followed by analysis with the general linear model with Statistical Analysis Systems (SAS; SAS Inst., Cary, NC, USA) version 9.1 [16]. All treatments were conducted in triplicate. Duncan's multiple range test and the orthogonal polynomial contrast were used to identify differences among the treatment groups. The differences were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The *in situ* rumen fermentation characteristics are shown in Table 2. The ruminal pH of the 15% RWR group decreased with time, and it was significantly higher ( $p < 0.01$ ) than that of the other treatments. In contrast, Jeong et al. [11] reported a higher ruminal pH ( $p < 0.05$ ) in the control group than in the 15% RWR group after 12 h of incubation. Alcohol promotes the fermentation of carbohydrates by rumen microorganisms, thereby increases the production of various fermented products. The total VFA concentration did not differ among the RWR-supplemented groups,

**Table 2.** Effects of rice wine residue (RWR) supplementation on the ruminal pH and volatile fatty acid (VFA) concentrations of Hanwoo steers

Parameter	Treatment <sup>1)</sup>			SEM	p-value
	CON	10% RWR	15% RWR		
pH	6.28 <sup>a</sup>	6.05 <sup>b</sup>	6.04 <sup>b</sup>	0.06	< 0.01
Total VFA (mM)	57.62	60.25	67.12	2.64	0.06
Acetate (mM)	29.23	29.71	32.94	1.16	0.12
Propionate (mM)	12.28	13.27	13.50	0.78	0.61
Butyrate (mM)	13.71 <sup>c</sup>	14.75 <sup>b</sup>	18.37 <sup>a</sup>	1.06	0.05
Valerate (mM)	0.42 <sup>c</sup>	0.49 <sup>b</sup>	0.64 <sup>a</sup>	0.04	< 0.01
iso-Butyrate (mM)	0.47	0.45	0.39	0.03	0.41
iso-Valerate (mM)	1.50	1.58	1.29	0.08	0.08
A:P ratio	2.39	2.24	2.44	0.11	0.95

<sup>1)</sup>TMR only (CON); TMR + 10% RWR (10% RWR); TMR + 15% RWR (15% RWR).

<sup>a-c</sup> Means in each row with different superscripts are significantly different ( $p < 0.05$ ).

RWR, Korean rice wine residue; VFA, volatile fatty acid; A:P, acetate/propionate ratio; TMR, total mixed ration.

but it tended to increase ( $p = 0.06$ ) in the 15% RWR group. The lower proportion of propionate among the VFAs during ethanol infusion can be attributed to one or more of the following: inhibition of propionate production during the metabolic process, synthesis of higher chain fatty acids such as valeric acid, and higher absorption of propionate through the rumen wall [17]. Liu et al. [18] reported that branched-chain volatile fatty acids (BCVFA) increased ruminal total VFA, acetate and butyrate, along with the enhanced fiber digestibilities, and microbial enzyme activities. Moreover, supplementation of BCVFA could promote the growth of calf by increasing ruminal butyrate production to improve the rumen development. The increased proportion of butyrate may also be related to an increase in acetate to butyrate conversion in the rumen [19–21]. Sutton et al. [21] reported that up to 64% of carbon in butyrate originated from acetate. A slight reduction in the proportion of acetate is possibly a result of increased conversion of acetate to butyrate [21]. A previous study showed that 28% of acetate in the rumen is not absorbed in the form of acetate [22]. Microorganisms can use acetate to produce butyrate through acetyl-CoA transferase and butyryl-CoA transferase [23]. Ruminal microbial metabolism through energy dissipation process continuously converts acetate into butyrate [23,24]. Moreover, acetate delivered by starch into the gastrointestinal tract stimulates the growth of butyrate producing bacteria, and enhances the acetate to butyrate conversion [25]. The results of *in vivo* total tract digestibility obtained in the present study are shown in Table 3. The DM digestibility in the 15% RWR group was significantly higher than that in the CON group ( $p < 0.05$ ), but it was not significantly different from that in the 10% RWR group (Table 3). These results are consistent with those of Smith et al. [26], who reported increased DM digestibility with WDGS treatment at different concentrations. Furthermore, Luebbe et al. [27] reported that rumen digestibility and total tract digestibility increased linearly with an increase in the WDG concentration. However, May et al. [28] reported that WDGS from corn and sorghum at different concentrations (from 0% to 30%, DM basis) did not affect the apparent total tract digestibility in feedlot steers.

The growth performance and blood profiles of Hanwoo steers fed TMR with or without RWR are presented in Tables 4 and 5, respectively. In the fattening stage, the total weight gain and ADG in the 10% RWR (322.86 and 0.82 kg, respectively) and 15% RWR groups (318.14 and 0.81 kg)



**Table 3.** Effects of rice wine residue supplementation on the total tract digestibility of Hanwoo steers

Digestibility (%)	Treatment <sup>1)</sup>			SEM	p-value
	CON	10% RWR	15% RWR		
Dry matter	71.71 <sup>b</sup>	73.90 <sup>ab</sup>	76.25 <sup>a</sup>	0.79	0.05
Crude protein	72.04	72.44	73.58	0.69	0.58
Ether extract	88.85	87.03	88.46	0.58	0.27
Neutral detergent fiber	64.19	62.23	64.17	1.23	0.71
Acid detergent fiber	55.16	55.07	57.61	1.31	0.66

<sup>1)</sup>TMR only (CON); TMR + 10% RWR (10% RWR); TMR + 15% RWR (15% RWR).

<sup>a,b)</sup>Means in each row with different superscripts are significantly different ( $p < 0.05$ ).

RWR, Korean rice wine residue; TMR, total mixed ration.

**Table 4.** Effects of rice wine residue supplementation on the growth performance of Hanwoo steers in the fattening stage

Parameter	Treatment <sup>1)</sup>			SEM	p-value
	CON	10% RWR	15% RWR		
Growth performance					
Initial body weight (kg)	336.16	350.33	338.40	9.76	0.06
Final body weight (kg)	609.54	673.19	656.54	18.15	0.35
Total weight gain (kg)	273.38 <sup>b</sup>	322.86 <sup>a</sup>	318.14 <sup>a</sup>	12.49	0.01
ADG (kg)	0.70 <sup>b</sup>	0.82 <sup>a</sup>	0.81 <sup>a</sup>	00.03	0.03
Feed intake (kg)	5,155.16 <sup>b</sup>	5,084.86 <sup>a</sup>	5,080.96 <sup>a</sup>	34.09	0.04
Feed efficiency	0.05 <sup>b</sup>	0.06 <sup>a</sup>	0.06 <sup>a</sup>	00.00	0.01

<sup>1)</sup>TMR only (CON); TMR + 10% RWR (10% RWR); TMR + 15% RWR (15% RWR).

<sup>a,b)</sup>Means in each row with different superscripts are significantly different ( $p < 0.05$ ).

RWR, rice wine residue; ADG, average daily gain; TMR, total mixed ration.

**Table 5.** Effects of rice wine residue supplementation on the blood profiles of Hanwoo steers in the fattening stage

Parameter	Treatment <sup>1)</sup>			SEM	p-value
	CON	10% RWR	15% RWR		
Ethanol (%)	0	0	0	0	-
Albumin (g/dL)	3.46	3.64	3.88	0.12	0.47
BUN (mg/dL)	13.38 <sup>a</sup>	11.78 <sup>b</sup>	11.26 <sup>b</sup>	0.84	0.04
Total protein (g/dL)	6.78 <sup>b</sup>	6.71 <sup>c</sup>	6.98 <sup>a</sup>	0.19	0.02
Creatinine (mg/dL)	0.75 <sup>b</sup>	0.91 <sup>ab</sup>	0.99 <sup>a</sup>	0.06	0.04
Glucose (mg/dL)	61.56	64.89	70.88	3.65	0.12
Total cholesterol (mg/dL)	101.78	117.44	124.38	6.75	0.87
Triglyceride (mg/dL)	15.56	20.78	22.25	3.63	0.75
LDL (mg/dL)	15.22	17.67	19.13	1.35	0.64
HDL (mg/dL)	88.22 <sup>b</sup>	102.55 <sup>ab</sup>	109.37 <sup>a</sup>	6.11	0.04
AST/SGOT (U/L)	60.56	60.56	59.88	3.95	0.11
ALT/SGPT (U/L)	24.11	22.67	25.25	1.83	0.18

<sup>1)</sup>TMR only (CON); TMR + 10% RWR (10% RWR); TMR + 15% RWR (15% RWR).

<sup>a-c)</sup>Means in each row with different superscripts are significantly different ( $p < 0.05$ ).

RWR, rice wine residue; BUN, blood urea nitrogen; LDL, low density lipoprotein; HDL, high density lipoprotein; AST/SGOT, aspartate aminotransferase/serum glutamic oxaloacetic transaminase; ALT/SGPT, alanine transaminase/serum glutamic pyruvate transaminase; TMR, total mixed ration.

were significantly higher ( $p < 0.05$ ) than those in the CON group (273.38 and 0.70 kg). These results suggest that a large amount of ethanol was oxidized to acetate by the rumen microorganisms; it was utilized for the synthesis of VFA of longer chain lengths [17]. Hanson and Ballard [29] reported that acetate is a major precursor for lipogenesis and that adipose tissue is considered the most important site for lipogenesis. Bulumulla et al. [30] reported that rumen VFAs might contribute to meat quality and carcass traits in beef cattle, and this supports the results of total weight gain and ADG in the present study. Yan [31] reported the beneficial effects of ethanol in improving the marbling score of Korean native steers. Furthermore, Lin [32] reported that the production of total volatile acids in the rumen increased whereas that of propionate decreased with the supplementation of alcohol-supplemented feed; this may improve body weight gain in Korean native steers through decreased protein degradation and increased fat synthesis [33]. Firkins et al. [34] fed animals WDG in the diet (50%) and observed a significant increase in the daily gain. The feed efficiency was significantly higher ( $p < 0.05$ ) in the 10% RWR (0.063) and 15% RWR groups (0.060) than in the CON group (0.053). Alcohol-fermented diets such as RWR are metabolized by the rumen bacteria [35], producing acetate [36] and other VFA. Furthermore, Durix et al. [37] reported that alcohol-fermented diets are degraded to VFAs by the rumen microorganisms. Our feed efficiency results are similar to those of Kristensen et al. [38], who varied the concentration of alcohol (8.7, 5.1, 3.8, and 5.2 MJ/d) in TMR and observed increased feed efficiency in lactating cows. Moreover, researchers have suggested that increased feed efficiency may be partially due to a reduction in subacute acidosis in cattle fed WDG [34,39]. Consequently, subacute acidosis reduces gain and feed efficiency [40] and nutrient absorption [41]. Ham et al. [42] reported that increased energy in distillers by-products, a possible reduction in subacute acidosis, a change in microbial population, and the effects of added moisture are key factors that may account for an increased feed efficiency in cattle. It has been reported that subacute acidosis alleviation is associated with an increase in feed intake [43,44].

Simion et al. [45] also reported normal concentrations of the GPT and GOT enzymes in the blood of cattle. Yan et al. [31] reported higher concentrations of serum triglycerides in steers fed alcohol-fermented feed. However, in this study, RWR did not affect the alcohol, albumin, glucose, total cholesterol, triglyceride, and LDL concentrations in the blood, and aspartate aminotransferase (AST)/SGOT and alanine aminotransferase (ALT)/SGPT activities; these parameters were within the normal range [46]. Low SGOT and SGPT activities are favorable for healthy cattle [46]. In the present study, the HDL and creatinine concentrations in the 15% RWR group (109.37 and 0.99 mg/dL, respectively) was significantly higher ( $p < 0.05$ ) than those in the other groups. Creatinine is produced as an end product of muscle metabolism and usually eliminated only via the kidneys [47]. Studies have reported that the creatinine concentration increases linearly with the body weight [48,49]. Therefore, the relationship between the creatinine concentration and body weight could follow the same relationship between muscle tissue or crude protein concentration and body weight [50]. These findings explain the increase in the creatinine concentration with RWR supplementation observed in the present study. Steers that showed a high weight gain also produced a high amount of creatinine after blood profile analysis, and this was supported by the findings of Schroeder et al. [48] and De Campeneere et al. [49]. Additionally, Kreul et al. [51] reported improved daily gain and feed efficiency in steers fed diet containing ethanol at 6% of the ration's DM. A higher energy level of alcohol-containing diets may partially account for the improvement in feedlot performance. Furthermore, Li et al. [52] reported that the addition of a suitable amount of alcohol to the diet of beef cattle can be effective in improving feed efficiency and meat quality. Moreover, Radosits et al. [53] and Smith [54] reported that the cattle blood serum creatinine concentration of up to 1.5 mg/dL is considered normal. Providing alcohol-fermented feed to ruminants increased

triglyceride, glucose, and cholesterol concentrations in the blood, and this increased the weight gain [33]. Body fat is correlated with HDL and LDL concentrations; as body weight or fat increases, the HDL and LDL concentrations also increase [46]. Cheeke et al. [55] reported that the designation of HDL is based on the density of blood lipoproteins to which the cholesterol is attached; proteins in solutions have a high density, and therefore HDL has a high protein content. It has been reported that feed concentrates should have a higher level of EE [56], and this supports the results of the present study. The CON group presented the highest ( $p < 0.05$ ) BUN concentration (13.58 mg/dL). The BUN concentration in cattle is mainly affected by dietary levels of energy and proteins [57]. Roseler et al. [58] suggested that the BUN concentration is an indicator of protein metabolism. The decrease in BUN concentration with the increase in RWR supplementation observed in the present study is supported by the results of Li et al. [52], who reported that 15% alcohol-fermented feed supplementation decreased the BUN concentration. In the present study, although the BUN concentration was significantly lower in the RWR-supplemented treatment groups, the values were still within the normal range. Miller et al. [59] reported that the normal BUN concentration in cattle ranges from 10 to 20 mg/dL. The blood total protein and albumin concentrations in both treatments were within the normal ranges [60]. Serum proteins can be used as an indicator of the nutritional status of an animal, and they constitute a portion of the amino acid pool of the body [61]. Overall, these results show that 15% RWR supplementation can be a substitute feed for ruminants, which will reduce feed costs and increase ADG and total gain.

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