

The Effects of Diets with Different Protein Contents on Growth Performance and Digestibility, and on Some Ruminal Fermentation and Blood Parameters, in Bafra Lambs ^{[1][2]}

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Abstract

The objectives of this study were to determine the effects of diet with increasing dietary levels of crude protein (CP) on digestibility, rumen pH, growth performance, volatile fatty acids (VFAs) and ammonia nitrogen (NH₃-N), and on several blood parameters (serum urea, glucose and total protein), in the finishing period of Bafra lambs. Thirty male Bafra lambs, which were 3-3.5 months of age and average live weight of 24±0.4 kg, were divided into three groups (n=10 lambs per group) in a completely randomized design experiment. The diets were composed of 80% compound feed containing 11, 14 or 17% CP and 20% vetch straw. Lambs were fed ad libitum twice each day during the feeding trial period of 60 days. In the last week of the feeding period, all feces were collected to determine digestibility. The rumen and blood samples were collected at the end of the feeding period. The average feed intake was similar among all tested diets. The best feed conversion rate was recorded the 17% CP diet. Final live weight and average daily gain (ADG) of lambs were significantly higher in lambs fed the 17% CP diet (P<0.05). CP digestibility was highest in the 17% CP diet (P<0.05) but the dry matter (DM) and organic matter (OM) digestibility were not affected by protein level. The rumen fluid NH₃-N level increased significantly (P<0.05), pH and butyric acid level were not significantly affected, ruminal acetic acid and acetate:propionate ratio increased significantly (P<0.05) and propionic acid level decreased significantly (P<0.05) with the increase in dietary CP level. No significant differences were observed between groups for serum glucose and albumin, while the serum urea levels in the 14% and 17% CP groups were higher than for 11% CP (P<0.05). The highest economic value was for the 17% CP at 187.97%. Bafra lambs fed a diet containing 17% CP and vetch straw achieved a significantly higher body weight, had the best FCR and yielded a higher net profit than the other groups. If larger scale, on-farm studies confirm the findings of this study, Bafra lamb producers should be encouraged to maximise potential net profit by adopting its feeding regime, including 17% CP.

Keywords: Bafra lambs, Crude protein level, Performance, Digestibility, Rumen fermentation, Blood parameters

Farklı Düzeylerde Protein İçeren Konsantre Yem Karışımının Bafra Irkı Kuzularda Besi Performansı, Sindirilebilirlik, Bazı Rumen ve Kan Parametrelerine Etkisi

Özet

Bu çalışmada, kuzu bitirme karma yemlerinde artan düzeyde ham proteinin (HP) Bafra kuzularında sindirilebilirlik, rumen pH'sı, büyüme performansı, uçucu yağ asitleri (UYA), amonyak azotu (NH₃-N) ve bazı kan parametreleri (serum üre, glukoz ve total protein) üzerine etkileri araştırıldı. Araştırmada, 3-3.5 aylık yaşta, ortalama 24±0.4 kg canlı ağırlığında 30 adet Bafra erkek kuzusu kullanıldı. Araştırmada kuzular her bir grupta 10 adet olacak şekilde rastgele 3 gruba dağıtıldı. Deneme gruplarında kullanılan konsantre yemin ham protein (HP) içeriği %11, 14 ve 17 olacak şekilde düzenlendi. Her bir deneme grubu rasyonun %80 konsantre yem ve %20 fiğ samanından oluşturuldu. Kuzular günde iki kez olacak şekilde 60 gün süre ile ad libitum beslendi. Tüm deneme grupları arasında ortalama yem tüketimleri benzer bulundu. En iyi yemden yararlanma oranı (5.19) %17 HP içeren konsantre yem tüketen grupta kaydedildi. Ortalama besi sonu canlı ağırlık ve günlük canlı ağırlık artışları %17 HP içeren bitirme yemlerini tüketen grupta istatistiksel olarak önemli derecede (p<0.05) yüksek bulundu. Rasyonda HP düzeyinin artması KM ve OM sindirilebilirliğini etkilemedi fakat %17 HP içeren grupta HP sindirilebilirliği önemli derecede yüksek saptandı (P<0.05). Rasyonda artan HP düzeyinin ruminal NH₃-N seviyesini yükselttiği (P<0.05), pH ve butirik asit seviyesini etkilemediği, asetik asit ve asetik asit:propionik asit oranını artırdığı (P<0.05), propionik asit oranını ise azalttığı (P<0.05) saptandı. Kan serum glikoz ve total protein düzeyi rasyonun HP düzeyinden etkilenmezken, üre düzeyi ise %14 ve 17 HP düzeylerinde %11 HP düzeyine göre yüksek bulundu (P<0.05). En yüksek ekonomik değere %187.97 ile %17 HP'li grupta saptandı. Bafra kuzularının %17 CP ve fiğ saman içeren rasyonla beslenmeleri, diğer gruplardan daha yüksek canlı ağırlık artışı, en iyi FCR ve net kâr sağlamıştır. Sonuç olarak, bu çalışmanın bulguları daha büyük ölçekli çiftlik çalışmaları ile desteklenirse, Bafra kuzusu yetiştiricileri maksimum net kar elde etmek için %17 HP içeren bitirme yemi ile besleme yapmaları yönünde teşvik edilebilir.

Anahtar sözcükler: Bafra kuzusu, Ham protein seviyesi, Besi performansı, Sindirilebilirlik, Rumen fermentasyonu, Kan parametreleri



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INTRODUCTION

In Turkey, which has a sheep population of approximately 31.50 million, the industry plays a crucial economic role^[1]. Productivity is not only hindered by feed of low quality and quantity but also the low genetic potential of sheep. The Bafra sheep is from a Sakız x Karayaka cross and is a better than either parent in terms of yield characteristics, including fertility, milk yield and live weight^[2]. This breed is widespread in the Black Sea Region of Turkey, especially in Samsun, Sinop, Ordu, Giresun, Tokat and Amasya Provinces. The majority of lambs born in spring and winter are fed under an intensive feeding system in order to meet the lamb demand in this region. The feeding of lambs on the basis of their nutritional requirements at different production stages is not a common system in Turkey. In addition, farmers tend to use feedstuffs with high energy content such as barley, corn or wheat in diets in order to obtain higher growth performance. Therefore, there is a need for supplemental protein to balance the diet and achieve optimum growth performance during the finishing period.

Balanced feeding in terms of energy and protein requirements optimizes animal growth and reproductive performance^[3]. Reynal and Broderick^[4] reported that a low protein diet may be unfavorable for microbial protein synthesis, ruminal digestion and the availability of nitrogen and energy. However, high levels of CP can lead to toxicity due to extreme release of ruminal ammonia^[5].

The National Research Council (NRC) recommends a diet containing 14.5% CP for the maximum growth of lambs that are weaned early^[6]. Several studies have been performed to determine the optimal dietary CP level for lambs. According to Andrew and Qrskov^[7], the maximum nitrogen retention and live weight gain of bred lambs grown from 15 to 40 kg on a high level of nutrition was achieved at 17% dietary CP. Though feeding lambs an 18% CP diet is conventional practice, there was no difference between lambs fed 16 and 18% CP which both had a significantly higher body weight gain and DM intake than lambs fed a 10, 12 or 14% CP diet. The authors also emphasised that there were no benefits of using diets with higher than 16% CP^[8-10]. Some studies have reported that DM and OM digestibility levels were not significantly different among different dietary levels of CP (10-17%), but CP digestibility levels were significantly higher in lambs feed higher CP levels^[9,11,12]. Rumen NH₃-N and VFA levels increased with increasing dietary CP level^[11,13-15]. Separately, it has been reported that there was no significant effect of dietary CP level on glucose and total serum protein levels, except for urea^[8,16].

Many studies have been conducted on the effects of different dietary protein levels on rumen fermentation, digestibility and growth performance in weaned lambs. However, there is no data on the nutritional requirements

specific to the Bafra lamb of Turkey. Therefore, the aim of this study was to investigate the effects of different dietary CP levels on rumen fermentation, digestibility and growth performance, and on some blood parameters, of weaned Bafra lambs.

MATERIAL and METHODS

The experiment was conducted at a private farm located in the Bafra district of Samsun Province, Turkey, from June 15 to July 16, 2016. The use and handling of the animals for this study was approved by the ethics council of Ondokuz Mayıs University (2015-08/71).

Animals, Diets and Experimental Procedures

Thirty male, Bafra lambs from 3 to 3.5 months of age and with mean, initial live weight of 24±0.4 kg were used in the experiment. The lambs were dewormed and vaccinated for common viral diseases before the trial which was conducted as a randomised block design. The thirty lambs were divided into three groups of 10 lambs. The diet with 11% CP mostly consisted of maize. Protein supplements were added to the other diets to obtain 14 and 17% CP levels. The diets were formulated to be nearly isocaloric (2700 kcal/kg). Concentrates were prepared in a commercial feed manufacturing factory as a mash feed. The diets of the three groups were composed of 80% compound feed which included 11, 14 or 17% CP (Table 1)

Table 1. Components and chemical composition of the experimental diets

Item	CP Levels (%)		
	11	14	17
Vetch straw	20.0	20.0	20.0
Maize	0	10.4	9.6
Barley	54	36.4	36.0
Wheat bran	20.8	16.8	8.8
Soybean meal	0	5.6	10.4
Canola meal	0	3.2	7.2
Molasses	3.2	5.6	6.0
Salt	0.8	0.8	0.8
Limestone	0.8	0.8	0.8
Vitamin and mineral mix ¹	0.4	0.4	0.4
Chemical composition			
CP (%)	11.3	13.7	16.0
Ruemen undegradable protein ² , (% of dietary CP)	22.5	27.5	29.4
ME ² (kcal/kg)	2.507	2.504	2.503

¹ 50.000 mg Mn, 50.000 mg Fe, 50.000 mg Zn, 10.000 mg Cu, 800 mg I, 150 mg Co, 200 mg Se, 50.000 mg Mg, 460.000 mg CaCO₃, 10.000 mg Antioxidant, 10.000.000 IU vitamin A, 2.000.000 IU vitamin D₃, 30.000 IU vitamin E; ² Calculated value

and 20% vetch straw. Fresh water was available to all animals at all times.

Lambs were fed as groups and were adapted to the feeding regime for 10 days before the experiment started. The experimental period for each group was 8 weeks. Diets were provided in equal amounts at 07:00 h and 16:00 h. The quantity of the daily diet was adjusted every day according to the previous day's feed intake by increasing at a rate 10%. Provided and refused feed were measured daily and mean feed intake was calculated as the difference between the two measurements divided by ten. The live weights of lambs were recorded before feeding in the morning on day one and on the final day (60th day) of the study.

For the *in vivo* digestibility trial, five lambs in each group were placed in individual pens at the end of the final week of the experiment. Total fecal output was determined for 5 days. Individual animal's feces was weighed daily and a 10% portion taken, homogenised and frozen. The homogenised feces were then dried in a forced air oven at 60°C for 48 h. DM, OM and CP digestibility were determined with the method of Kaya et al.^[11].

On the final day of the experiment, rumen fluid samples were collected in tubes from seven lambs from each group with the aid of an oral stomach tube, 2 h after the morning feeding. The samples were collected in a manner that ensured that they were not contaminated with saliva. The pH was measured immediately with a pH meter. Samples were then filtered through four layers of cheesecloth to remove the solid, unfermented particles. For VFA analysis, a 10 mL aliquot of ruminal fluid was acidified with 2 mL of 25% metaphosphoric acid and centrifuged at 5.000 rpm for 10 min. A 1.5 mL portion of the supernatant was frozen at -20°C for subsequent analyses^[17]. In addition, 10 mL subsamples of the strained rumen fluid were preserved by the addition of 0.2 mL 50% H₂SO₄ to stop bacterial activity and preserve the NH₃-N, and stored at -20°C until analysis^[18].

At the end of the feeding trial, blood samples were collected immediately before the morning feeding from each lamb via jugular venipuncture into non-heparinized tubes. The samples were centrifuged at 1.000 × g for 15 min at 4°C. The serum was separated from the collected blood, then transferred into a polypropylene tube and stored at -18°C until analysis.

Chemical Analyses

Samples of the feed rations and the feces were used to determine DM, OM, CP and ash, according to the methods described by the AOAC^[19]. Rumenal NH₃-N was determined with the macro-Kjeldahl procedure^[19]. For VFA analysis, a method was used as described previously^[20] with some modifications, by using a gas chromatograph (GC 17A,

Shimadzu, Kyoto, Japan) equipped with a Flame Ionisation Detector (FID). Just before analysis, the rumen samples were thawed and centrifuged at 4.000 g at 0°C for 30 min. The clear supernatant was diluted by a factor of 10 in ultrapure water, then filtered through a 0.45 µm PVDF disk filter before injection into the gas chromatograph. A TRB-FFAP capillary column (30 m×0.25 mm×0.25 µm, Teknokroma, Spain) was used. The carrier gas was nitrogen at a flow rate 5.05 ml/min; the column oven temperature range was 60-200°C (20°C increase/min), then held for 5 min at 200°C (total analysis time: 12 min); the injector temperature was 250°C; and the detector temperature was 300°C. The 1 µL samples were injected into the GC system with an autosampler (AOC 5000 plus, Shimadzu, Kyoto, Japan) at a 1:20 splitting ratio. Serum blood samples were analysed for total protein, glucose and urea concentration by using an autoanalyzer (Mindray BS120).

Economic Analysis

Economic analysis of the data was performed with the method of Mirza et al.^[21]. The cost of an 11% CP diet was taken to be 0.845 TRY/kg, that of the 14% CP diet was 0.925 TRY/kg, that of the 17% CP diet was 1 TRY/kg, and that of vetch straw was 0.4 TRY/kg, in 2016. 1 US Dollar was valued at about 2.97 TRY in September, 2016 and the live weight value was 14 TRY/kg.

Statistical Analyses

Date regarding various parameters were analysed using one-way ANOVA in the SPSS 21.0 software package^[22]. Differences were considered significant at 5% (P<0.05), and the comparison of means was carried out with the Duncan test.

RESULTS

Growth Performance and Digestibility

The effects of CP level in the diet on growth performance, digestibility, ruminal fermentation and blood parameters, and the economic analysis, are shown in *Tables 2, 3, 4, and 5*. Increased dietary protein increased feed consumption, final live weight, average daily gain (ADG) and FCR (*Table 2*). The lowest feed consumption was observed in the 14% CP group but it was not evaluated statistically because lambs were fed by group in the experiment. Final live weight and ADG were significantly higher for the 17% CP diet than for the 11 and 14% CP diets (P<0.05). At the end of the feeding trial, the average live weights for the 11, 14 and 17% CP groups were 33.66, 36.38 and 38.77 kg, respectively. ADG was highest (P<0.05) in lambs fed 17% CP (241.81 g), followed by 14% CP (213.58 g) and 11% CP (156.16 g). The average daily feed consumption in the 11, 14 and 17% CP groups was 1221.7 1215.2 and 1254.1 respectively. The highest and the lowest FCRs were observed in the 17 and 11% CP groups, respectively. Although there was

Table 2. Effect of CP levels on growth performance and digestibility

Item	CP Levels (%)			P
	11	14	17	
Initial weight (kg)	24.29±0.33	24.07±0.40	24.27±0.35	NS
Final weight (kg)	33.66±0.39 ^c	36.88±0.65 ^b	38.77±0.28 ^a	*
Average daily gain (g)	156.16±2.40 ^c	213.58±4.62 ^b	241.81±4.07 ^a	*
Feed intake (g/day) ¹	1221.78±21.89	1215.25±31.39	1254.15±25.63	
Feed conversion ratio ²	7.35	5.66	5.19	
Digestibility (%)				
DM	74.61±0.77	75.28±0.53	74.83±0.95	NS
OM	73.19±1.02	74.37±1.69	73.50±2.49	NS
CP	70.54±1.91 ^c	74.01±2.92 ^b	78.73±0.59 ^a	*

^{a,b,c}The groups in the same row labeled different letters are statistically significant ($P<0.05$)
¹ it was not evaluated statistically because lambs were fed by group in the experiment; ² Feed conversion ratio= (g feed intake/g body weight gain); NS: Non-significant, * $P<0.05$

Table 3. The effect of protein levels on ruminal pH, NH₃-N and VFA concentration

Item	CP Levels (%)			P
	11	14	17	
pH	6.47±0.7	6.54±0.08	6.51±0.04	NS
NH ₃ -N (mmol L ⁻¹)	131.5±6.85 ^c	157.00±6.39 ^b	179.42±3.41 ^a	*
VFA (mmol L⁻¹)				
Acetic acid	53.02±0.64 ^c	56.81±0.41 ^b	59.91±1.06 ^a	*
Propionic acid	27.68±0.37 ^a	25.35±0.33 ^b	22.69±1.03 ^c	*
Butyric acid	12.87±0.23	13.20±0.20	13.14±0.18	NS
Acetate:propionate	1.91±0.02 ^c	2.24±0.76 ^b	2.66±0.09 ^a	*

^{a,b,c}The groups in the same row labeled different letters are statistically significant ($P<0.05$)
NS: Non-significant, * $P<0.05$, VFA: Volatile fatty acid

Table 4. The effects of CP levels on some blood parameters

Item	CP Levels (%)			P
	11	14	17	
Total protein (g/dL)	6.22±0.29	6.73±0.27	6.86±0.19	NS
Glucose (mg/dL)	76.63±0.45	76.31±0.36	76.29±0.33	NS
Urea (mg/dL)	33.36±0.57 ^b	51.35±0.72 ^a	54.23±0.91 ^a	*

^{a,b}The groups in the same row labeled different letters are statistically significant ($P<0.05$)
NS: Non-significant, * $P<0.05$

no significant difference between the groups in terms of DM and OM digestion, the CP digestion level increased significantly with the dietary protein level ($P<0.05$).

Rumen Parameters

The rumen fermentation parameters are presented in Table 3. The NH₃-N level significantly increased with CP content ($P<0.05$) but ruminal pH values were not significantly affected by CP content. VFA concentrations, except for butyric acid, were significantly affected by dietary protein

level ($P<0.05$). The molar percentage of propionic acid decreased linearly ($P<0.05$) with increasing protein level, while the acetic acid concentration increased ($P<0.05$). Increasing the CP level from 11 to 17% caused the acetate to propionate ratio to significantly increase ($P<0.05$) from 1.91 to 2.66.

Blood Parameters

Serum urea levels of the 14 and 17% CP groups were similar and significantly higher than for the 11% CP group

Table 5. Economic analysis of live weight gain			
Item	CP Levels (%)		
	11	14	17
Feed intake			
Protein supplement intake (g/head/day)	990	988	1022
Straw intake (g/head/day)	231	226	232
Total feed intake (supplement+straw)(g/head/day)	1221	1215	1254
Cost of feed intake¹ (TRY/head/day)			
Protein supplement	0.83	0.91	1.022
Straw	0.09	0.09	0.09
Total cost	0.92	1.0	1.11
Average liveweight gain (g/head/day)	156	213	254
² Total benefit of liveweight gain at TRY/14/kg (TRY/head/day)	2.18	2.98	3.55
Net benefit (TRY/head/day)	1.26	1.98	2.44
¹ 11% CP concentrate 0.845 TRY/kg, 14% CP concentrate 0.925 TRY/kg, 17% CP concentrate 1.0 TRY/kg, straw: 0.4 TRY/kg in year 2016			
² Price of one kilogram live body of lamb was 14 TRY in year 2016			

($P < 0.05$) but there were no significant differences in serum TP and glucose levels for lambs fed 11, 14 or 17% CP (Table 4).

Economic Analysis

The economic analysis is shown in Table 5. The net benefit of diets with different protein contents was calculated by giving a value of 14 TRY per kilogram of body weight gain and subtracting the expenditure on feed from that value. The net benefit was highest in lambs fed 17% CP (2.44 TRY/day), followed by 14% CP (1.98 TRY/day) and 11% CP (1.26 TRY/day).

DISCUSSION

In this study, the feed intake of the three experimental groups ranged from 1.215 to 1.254 g/day. These results support those of Bilal et al.^[8] who reported that the lowest dry matter intake was observed at the 10% CP level. Also, many researchers have reported no effect of dietary CP content on DMI^[23,24]. In contrast, Drouillard et al.^[25] reported a 7% increase in DMI when lambs were fed a 14.5% CP diet compared with an 8.9% CP diet. Furthermore, Fluharty and McClure^[26] demonstrated an increase in DMI when lambs were fed a high protein diet (18.9% CP) compared with a 14.5% CP diet. Also, Haddad et al.^[9] reported an incremental increase in DMI as dietary CP content increased, being highest for 16 and 18% CP diets. In the present study, the 17% CP diet resulted in the best FCR, followed by 14% CP and 11% CP. The better FCR attained by feeding the 17% CP diet may have been due to the more efficient utilization of protein and metabolism of energy sources for growth. These results are similar to the findings of Mahmoud^[23], who demonstrated a significantly higher FCR for 14% and 17% CP diets than for an 11% CP diet. Also, studies by

Kebede^[27] and Abebe et al.^[28] reported an improvement in the feed utilization and growth performance of Arsi-bale lambs at higher protein levels.

In the current study, the average final weight was highest ($P < 0.05$) for the 17% CP diet, followed by 14 and 11% (Table 2). Improved growth performance was also reported at higher dietary CP levels (16 to 18%) than at lower CP levels (10 to 14%) by Awassi^[9,29] and in Kivircik lambs^[8]. Similarly Kaya et al.^[11] and Abbasi et al.^[30] reported higher growth for Morkaraman and Kooka lambs, respectively, at higher CP (16%) than at a lower level of dietary CP.

In contrast to the current study, Dabiri and Thonney^[12] stated that there was no difference in live weight gain between groups fed 13, 15 or 17% CP for 42 days. Also, in a study performed on Arsi-bale lambs fed different levels of CP (9.4, 10.2, 11, 12 and 13.1%), there was no difference in final live weight^[27]. In the present study, the differences in final weight are attributable to differences in the mean daily weight gain of the lambs. ADG was higher in lambs fed with 17% dietary CP diet (241.81 g) than 14% (213.58 g) ($P < 0.05$), followed by 11% CP (156.16 g) (Table 2). The highest mean daily gain and final live weight obtained with the 17% CP diet may have been due to the increased CP creating a better balanced diet with a more appropriate ratio of energy and digestible protein for growing lambs. Furthermore, the higher grow rate or live weight gain with 17% CP diet may be due to higher amount of RUP content and amino acid profile of soya bean meal and canola meal.

The DMs and OM_s of the three dietary CP levels were not significantly different (Table 2), but the digestibility of CP in the 11% CP group was significantly lower than that of other treatments ($P < 0.05$). The higher CP digestibility for the lambs that were fed 17% CP supports the findings of higher digestibility of CP and rumen degradable protein

(RDP) in diets with the highest level of CP [29]. Similarly, Kaya et al.^[11] reported no significant difference between groups for DM and OM digestibility but the CP digestibility level significantly increased with increasing dietary protein level. Moreover, these results are similar to the findings of Dabiri and Thonney^[12] who found no differences in DM and OM digestibility levels but higher CP digestibility in the 17% CP group than in the 13% CP group. Contrary to our results, there are other studies reported that dietary CP content increases DM and OM digestion^[9,31] or that CP does not affect digestion efficiency^[32]. In the current study, it is possible that the lowest CP level (11%) limited the population growth and total activity of rumen microbes, whereas the higher CP levels may have induced increased microbial protein synthesis and fermentation, as suggested by Sharifi et al.^[32].

In the current study, the ruminal pH value was not significantly affected by increasing CP level ($P > 0.05$). pH values appear to be optimum between 6.5 and 7 for the microbial digestion of cellulose and protein^[33]. This may have been due to increased rumination during the night, roughage intake in the early mornings, and nitrogen influx into the rumen when nitrogen concentrations were low^[34]. The results of the present study are in agreement with several *in vitro* studies^[15,34-36]. In the present study, each increase in the level of dietary CP was accompanied by a significantly higher ruminal $\text{NH}_3\text{-N}$ concentration ($P < 0.05$). Similar results were reported by Sarwar et al.^[13], Abadi et al.^[14] and Yang et al.^[15]. However, Dutta et al.^[3], Vosooghi-poostindoz et al.^[35] and Chanthakhoun and Wanapat^[37] reported that increasing the CP level from 12 to 14.1% had no significant effect on the $\text{NH}_3\text{-N}$ level in the rumen; they all concluded that this could have been due to a higher level of incorporation of $\text{NH}_3\text{-N}$ in the microbial protein. Difference in the CP content among treatments is the primary reason for the observed differences in ruminal $\text{NH}_3\text{-N}$ concentration^[33]. In the present study, the increase in rumen $\text{NH}_3\text{-N}$ is probably due to an increase in proteolysis, the degradation of peptides and the deamination of amino acids in the rumen, as reported by Sharifi et al.^[32].

The level of VFA production in the rumen is influenced by the carbohydrate fraction and degradability^[38]. In the present study, the molar concentration of acetate and the acetate/propionate ratio in the ruminal fluid of Bafra lambs fed with a 17% CP diet were significantly higher than for those fed with 11 and 14% CP diets (Table 3) ($P < 0.05$), which might be due to the availability of higher amounts of protein in the diet promoting the growth of ruminal bacteria that subsequently produce acetate by degrading cellulose^[37]. In addition, higher feed intake of vetch straw in 17% CP group may lead to change of VFA concentration. In contrast, the propionate concentration was higher when lambs were fed a diet with lower CP (11%). Considering that the ruminal concentration of VFAs

at a particular time is the net result of their production and absorption, the lower propionate concentration in the other two groups (14 and 17% CP) in the present study may be explained by the higher proportion of barley (54%) in the 11% CP diet. It has been reported that corn is a fermentation substrate for the VFAs and therefore may increase propionate production when the lambs are fed with a lower CP content^[15]. The results of our study contradicted the results of two studies in which the effects of different protein levels on rumen fermentation were investigated^[11,14] but similar results were reported by Yang et al.^[15] who stated that lambs fed diets with increased protein levels had a lower propionate and higher acetate concentration.

Diseases and nutritional disorders can induce changes in blood parameters. The evaluation of the effects of dietary CP level on blood metabolites is therefore important^[8]. The normal serum TP^[39] and glucose^[8] levels in sheep range between 5.5 and 7.5 and 44.0 and 81.2 mg/dL, respectively. In the current study, serum TP and glucose levels were in the normal range and there were no significant differences in TP and glucose levels in lambs fed with an 11, 14 or 17% CP diet (Table 4). These results were compatible with those obtained by Keser et al.^[8], Shalu et al.^[16] and Hatfield et al.^[40]. Urea is a key metabolite produced from ammonia in the liver during protein metabolism^[8]. The levels of plasma urea and the dietary protein level were correlated in sheep^[41]. The normal urea levels of lambs was reported as 20-30 mg/dL^[42]. The urea levels in the present study were higher than the normal range (Table 4). However, the increases in serum urea levels in the present study due to an increased dietary protein level are in a line with the results of previous studies^[8,16,40,43].

In the present study, the cost-benefit analysis demonstrated a highest economic benefit of 2.44 TRY/day for the 17% CP diet, followed by 1.98 TRY/day for the 14% CP diet and 1.26 TRY/day for the 11% CP diet. The differences in economic benefit among the groups was due to the extra live weight gain attributable to the increase in the protein level of the diet. Similar results have been found in many studies conducted with lambs of various breeds fed different protein levels^[30,44].

In the current study, Bafra lambs fed a diet containing 17% CP achieved the highest live weight and FCR and generated the highest net profit. It is therefore concluded that Bafra lamb producers should be encouraged to maximise the potential net profit by adopting the 17% CP feeding regime used in the present study, if the results of the current study are confirmed by a larger scale, on-farm study.

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REFERENCES

- TUIK - Turkish Statistical Institute:** Statistical Database. Accessed: May 15, 2016.
- Arıtürk E, Akçapınar H, Aydoğan M, Bayrak S:** Karayaka koyun irkinin melezieme ile islahi. *Doğa Turk Vet Hay Derg*, 11, 21-26, 1987.
- Dutta TK, Agnihotri MK, Sahoo PK, Rajkumar V, Das AK:** Effect of different protein-energy ratio in pulse by-products and residue based pelleted feeds on growth, rumen fermentation, carcass and sausage quality in Barbari kids. *Small Ruminant Res*, 85, 34-41, 2009. DOI: 10.1016/j.smallrumres.2009.07.002
- Reynal SM, Broderick GA:** Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. *J Dairy Sci*, 88, 4045-4064, 2005. DOI: 10.3168/jds.S0022-0302(05)73090-3
- Chanthakhoun V, Wanapat M, Berg J:** Level of crude protein in concentrate supplements influenced rumen characteristics, microbial protein synthesis and digestibility in swamp buffaloes (*Bubalus bubalis*). *Livest Sci*, 144, 197-204, 2012. DOI: 10.1016/j.livsci.2011.11.011
- NRC:** Nutrient requirement of sheep. 6th edn., National Research Council, National Academy Press, Washington, DC, USA, 1985.
- Andrews RP, Orskov ER:** The nutrition of early weaned lamb. I. The influence of protein concentration and feeding level on rate of gain in body weight. *J Agric Sci (Camb)*, 75, 11-18, 1970. DOI: 10.1017/S0021859600025995
- Keser O, Bilal T:** Effect of different dietary protein levels on performance, N digestibility and some blood parameters in Kivircik lambs. *Acta Vet (Beograd)* 58, 487-498, 2008. DOI: 10.2298/AVB0806487K
- Haddad SG, Nasr RE, Muwalla MM:** Optimum dietary crude protein level for finishing Awassi lambs. *Small Ruminant Res*, 39, 41-46, 2001. DOI: 10.1016/S0921-4488(00)00172-3
- Titi HH, Tabbaa MJ, Amasheh MG, Barakeh F, Daqamseh B:** Comparative performance of Awassi lambs and Black goat kids on different crude protein levels in Jordan. *Small Ruminant Res*, 37, 131-135, 2000. DOI: 10.1016/S0921-4488(99)00136-4
- Kaya I, Unal Y, Sahin T, Elmali D:** Effect of different protein levels on fattening performance, digestibility and rumen parameters in finishing lambs. *J Anim Vet Adv*, 8, 309-312, 2009.
- Dabiri N, Thonney ML:** Source and level of supplemental protein for growing lambs. *J Anim Sci*, 82, 3237-3244, 2004. DOI: 10.2527/2004.82113237x
- Sarwar M, Nisa M, Javaid A, Shahzad MA:** Ruminant characteristics, blood pH, blood urea nitrogen and nitrogen balance in Nili-Ravi buffalo (*Bubalus bubalis*) bulls fed diets containing various level of ruminally degradable protein. *Ital J Anim Sci*, 6, 441-445, 2010. DOI: 10.4081/ijas.2007.s2.441
- Ibrahimi Khoram Abadi E, Tahmasebi AM, Danesh Mesgaran M, Naserian AA, Vakili A:** Effect of dietary crude protein level on UT-B expression and nitrogen efficiency in growing Baluchi male lambs fed low or high concentrate diets. *Iran J Appl Anim Sci*, 5, 323-332, 2015.
- Yang C, Bing-wen SI, Qi-yu D, Hai J, Shu-qin Z, Yan T:** Rumen fermentation and bacterial communities in weaned Chahaer lambs on diets with different protein levels. *J Integr Agric*, 15, 1564-1574, 2016. DOI: 10.1016/S2095-3119(15)61217-5
- Shalu T, Hart SP, Fernandez JM:** Nitrogen metabolism and blood metabolism in three goat breeds fed increasing amounts of protein. *Small Ruminant Res*, 10, 281-92, 1993. DOI: 10.1016/0921-4488(93)90133-3
- Erwin ES, Marco GJ, Emery EM:** Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. *J Dairy Sci*, 44, 1768-1771, 1961. DOI: 10.3168/jds.S0022-0302(61)89956-6
- Kazemi-Bonchenari MK, Rezayazdi K, Nikkhah A, Kohram H, Dehghan-Banadaky M:** The effects of different levels of sodium caseinate on rumen fermentation pattern, digestibility and microbial protein synthesis of Holstein dairy cows. *Afr J Biotechnol*, 9, 1990-1998, 2010. DOI: 10.5897/AJB09.1325
- AOAC:** Official Methods of Analysis, 18th edn., Association of Official Analytical Chemists, Inc., Arlington, VA, 2006.
- Filipek J, Dvorak R:** Determination of the volatile fatty acid content in the rumen liquid: Comparison of gas chromatography and capillary isotachopheresis. *Acta Vet Brno*, 78, 627-633, 2009. DOI: 10.2754/avb200978040627
- Mirza IH, Anjum MI, Khan AG, Azim A:** Comparative evaluation of cotton seed meal and sunflower seed meal in urea molasses blocks versus commercial concentrate as supplement to basal ration of wheat straw with stall-fed Buffalo calves. *Asian-Aust J Anim Sci*, 17, 193-198, 2004. DOI: 10.5713/ajas.2004.193
- SPSS:** Statistical Packages for Social Science. Version 21, SPSS In., Illionsa, USA, 2007.
- Mahmoud AEM:** Impact of dietary protein levels on digestibility, blood parameters, insulin like growth factore-1 and growth performance of growing rahmani lambs. *Egypt J Nutr Feeds*, 16, 195-202, 2013.
- Beauchemin KA, McClelland LA, Kozub GC, Jones SDM:** Effects of crude protein content, degradability and energy concentration of the diet on growth and carcass characteristics of market lambs fed high concentrate diets. *Can J Anim Sci*, 75, 387-395, 1995. DOI: 10.4141/cjas95-058
- Drouillard JS, Klopfenstein TJ, Britton RA, Bauer ML, Gramlich SM, Wester TJ, Ferrell CL:** Growth, body composition, and visceral organ mass and metabolism in lambs during and after metabolizable protein or net energy restriction. *J Anim Sci*, 69, 3357-3375, 1991. DOI: 10.2527/1991.6983357x
- Fluharty FL, McClure KE:** Effect of dietary energy intakes and protein concentration on performance and visceral organ mass in lambs. *J Anim Sci*, 75, 604-10, 1997. DOI: 10.2527/1997.753604x
- Kebede G:** Optimum dietary crude protein level for fattening yearling Arsi-Bale lambs. *World J Agric Sci*, 11, 101-106, 2015.
- Tafa A, Melaku S, Peters KJ:** Supplementation with linseed (*Linum usitatissimum*) cake and/or wheat bran on feed utilization and carcass characteristics of Arsi-Bale sheep. *Trop Anim Health Prod*, 42, 677-685, 2010. DOI: 10.1007/s11250-009-9475-8
- Santos RS, Ribeiro KG, Valadares Filho SC, Pereira OG, Villela SDJ, Silva JL:** Effects of diets with high and low protein contents and two concentrate levels in Santa Ines x Texel lambs. *Livest Sci*, 177, 79-87, 2015. DOI: 10.1016/j.livsci.2015.04.011
- Abbasi IHR, Sahito HA, Abbasi F, Menghwar DR, Kakal NA, Sanjrani IG:** Impact of different crude protein levels on growth of lambs under intensive management system. *Int J Adv Res*, 2, 227-235, 2014.
- Mawati S, Soedarsono S, Sunarso S, Purnomoadi A:** The Effects of different energy and protein ratio to sheep's nutrient intake and digestibility. *ISJE*, 4, 76-79, 2013. DOI: 10.12777/ijse.4.2.75-79
- Sharifi M, Bashtani M, Naserian AA, Khorasani H:** Effect of dietary crude protein level on the performance and apparent digestibility of Iranian Saanen kids. *Afr J Biotechnol*, 12, 4202-4205, 2013.
- Wanapat M, Cherdthong A:** Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in Swamp buffalo. *Curr Microbiol*, 58, 294-299, 2009. DOI: 10.1007/s00284-008-9322-6
- Bae DH, Welch JG, Smith AM:** Forage intake and rumination by sheep. *J Anim Sci*, 49, 1292-1299, 1979. DOI: 10.2527/jas1979.4951292x
- Vosooghi-poostindoz V, Foroughi AR, Delkhoroshan A, Ghaffar MH, Vakili R, Soleiman AK:** Effects of different levels of protein with or without probiotics on growth performance and blood metabolite responses during pre- and post-weaning phases in male Kurdi lambs. *Small Ruminant Res*, 117, 1-9, 2014. DOI: 10.1016/j.smallrumres.2013.11.015
- Chen S, Paengkoum P, Xia X, Na-Lumpang P:** Effects of dietary protein on ruminal fermentation, nitrogen utilization and crude protein maintenance in growing Thai indigenous beef cattle fed rice

straw as roughage. *J Anim Vet Adv*, 9, 2396-2400, 2010. DOI: 10.3923/javaa.2010.2396.2400

37. Chanthakhoun V, Wanapat M: Effect of legume (*Phaseolus calcaratus*) hay supplementation on rumen cellulolytic bacterial populations in swamp buffaloes investigated by the real-time PCR technique. *J Anim Vet Adv*, 9, 1654-1659, 2010. DOI: 10.1016/j.livsci.2011.02.003

38. Mc Donald P: Animal Nutrition. Pearson Education, Prentice Hall, Harlow, 2002.

39. MVM: The Merck Veterinary Manual. 7th edn., Merck & Co, Inc, USA, 1991.

40. Hatfield PG, Hopkins JA, Ramsey WS, Gilmore A: Effects of level of protein and type of molasses on digesta kinetics and blood metabolites in sheep, *Small Ruminant Res*, 28, 161-170, 1998. DOI: 10.1016/S0921-4488(97)00085-0

41. Hammond AC: Effect of dietary protein level, ruminal protein solubility and time after feeding on plasma urea nitrogen and the relationship of plasma urea nitrogen to other ruminal and plasma parameters. *J Anim Sci*, 57, 435, 1983.

42. Bilal T: Veteriner Hekimlikte Laboratuvar Tanı. İstanbul Üniversitesi Basım ve Yayınevi Müdürlüğü, İstanbul, 2004.

43. Yurtman İY, Savas T, Karaagac F, Coskuntuna L: Effects of daily protein intake levels on the oral stereotypic behaviours in energy restricted lambs. *Appl Anim Behav Sci*, 1889, 1-12, 2002. DOI: 10.1016/S0168-1591(02)00025-4

44. Shahbabak MS, Rozbahan Y, Shahrabak MM, Shahrabak HM: Effect of different levels of digestible undegradable protein on the fattening performance of Kermani male lambs. *Int J Agri Biol*, 8, 721-723, 2006.