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Research Article

The Effect of Dietary Protein And Tannin Level on Fattening Performance, Rumen Volatile Fatty Acid Profile and Methane Production in Lambs

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ABSTRACT
The aim of this study was to evaluate two protein levels (14% and 18%) and two tannin levels (0 and 4%) in fattening meat-type lambs. The total of thirty-six Çukurova Meat Type lambs (3/8 Rambouillet, 3/8 Ile de France, 1/8 Awassi, 1/8 Chios) having 24.69±0.87 kg initial live weight divided into 4 groups having 9 male lambs with three replicates consisting three lambs in each. The total mixed rations for each treatment were prepared using 10% chopped alfalfa hay and 90% concentrate, ensuring they were isocaloric. Daily weight gain and feed conversion ratios were not affected by any of the factors (P>0.05). However feed intake was significantly affected by protein level and interaction between protein and tannin level (P<0.01). The factors did not significantly affect the concentrations of acetic and propionic acids in rumen fluid (P > 0.05). However, butyric and valeric acids were influenced by tannin level and the protein x tannin interaction (P < 0.05). Isobutyric acid was influenced by both protein and tannin levels, as well as their interaction (P < 0.05). Increase in protein level caused an increase in isobutyric acid, while addition of tannin to the diet decreased isobutyric acid in the rumen fluid. The decrease in isobutyric acid was more pronounced in the high protein diet compared to the low-protein diet. Isovaleric acid was significantly affected by dietary protein level (P < 0.01), with lambs receiving a high-protein diet having a higher concentration (0.32 vs. 0.67 mmol/L) compared to those on a low-protein diet. Total 24-hour gas production was affected by the interaction between protein and tannin levels, while 24-hour methane production was affected by all factors (P < 0.05). Tannin decreased total gas production in the low-protein diet but increased it in the high-protein diet. Similarly, tannin did not affect 24-hour methane production in the low-protein diet but increased methane production in the high-protein diet. In conclusion, lambs with an initial body weight of 25 kg did not benefit from an increase in dietary protein or the inclusion of tannins in their diet. However tannin decreased protein degradation when the isoacids production was evaluated.

Keywords: lamb, tannin, protein, interaction, volatile fatty acids

Kuzularda Diyetle Verilen Protein ve Tanen Düzeyinin Besi Performansı, Rumen Uçucu Yağ Asidi Profili ve Metan Üretimi Üzerine Etkisi

ÖZ
Bu çalışmanın amacı, etçi tip kuzularda iki farklı protein düzeyi (%14 ve %18) ile iki farklı tanen düzeyini (%0 ve %4) test etmektir. Toplam 36 Çukurova Etçi Tip kuzu (3/8 Rambouillet, 3/8 Île-de-France, 1/8 Awassi, 1/8 Sakız) 24,69 ± 0,87 kg canlı ağırlıkta başlangıç yapmış, her biri 9 erkek kuzudan oluşan 4 gruba ayrılmış ve her grupta 3 kuzudan oluşan 3 tekerrür bulunmuştur. Rasyonlar izokalorik olarak düzenlenmiştir. Her deneme yemi, %10 kıyılmış yonca otu ve %90 konsantre yem içeren toplam karışık rasyon şeklinde hazırlanmıştır. Günlük canlı ağırlık artışı ve yem dönüşüm oranları hiçbir faktörden etkilenmemiştir (P > 0,05), ancak yem tüketimi protein düzeyi ile protein × tanen düzeyi etkileşiminden etkilenmiştir (P < 0,01). Rumen sıvısında asetik ve propiyonik asitler üzerinde faktörlerin anlamlı etkisi görülmemiştir (P > 0,05). Buna karşılık bütirik ve valerik asitler, diyetin tanen düzeyi ile protein × tanen düzeyi etkileşiminden etkilenmiştir (P < 0,05). İzobütirik asit hem ana faktörlerden hem de etkileşimlerinden etkilenmiştir (P < 0,05). Protein düzeyindeki artış izobütirik asit miktarını artırırken, diyete tanen ilavesi izobütirik asit düzeyini düşürmüştür; bu azalma

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yüksek proteinli diyette daha belirgin, düşük proteinli diyette ise daha hafif olmuştur. Rumen sıvısındaki izovalerik asit, diyet protein düzeyinden etkilenmiştir ($P < 0,001$); yüksek proteinli diyet alan kuzularda konsantrasyon daha yüksek bulunmuştur (0,32 mmol/L'ye karşı 0,67 mmol/L). 24 saatlik toplam gaz üretimi protein × tanen düzeyi etkileşiminden, 24 saatlik metan üretimi ise tüm faktörlerden etkilenmiştir ($P < 0,05$). Tanen, düşük proteinli diyette toplam gaz üretimini azaltmış, ancak yüksek proteinli diyette artırmıştır. Benzer şekilde, tanen düşük proteinli diyette 24 saatlik metan üretimini değiştirmezken, yüksek proteinli diyette artırmıştır. Sonuç olarak, başlangıç canlı ağırlığı 25 kg olan kuzular, diyet protein düzeyinin artırılmasından ve diyette tanen kullanımından fayda görmemiştir. Bununla birlikte, izoasit üretimleri değerlendirildiğinde tanen, protein yıkımını azaltmıştır.
Anahtar kelimeler: kuzu, tannin, protein, interaksiyon, uçucu yağ asitleri

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Introduction

Tannins are water-soluble polyphenolic compounds with highly variable chemical structures, found in plants such as chestnut, oak, acorn, and sumac. These phenolic compounds are produced by plants to protect themselves from pathogenic microorganisms and viruses (Foley et al., 1999). It has had a wide range of uses since ancient times, such as medicine, cosmetics and the food industry. In recent years, the use of natural additives in animal nutrition has increased and tannins have become a remarkable research topic in this context. Tannins can form insoluble complexes with macromolecules, such as proteins, carbohydrates, and minerals (Swain, 1979). Tannins are divided into two main groups: condensed and hydrolyzed tannins. Hydrolyzed tannins are formed by the esterification of gallic acid or ellagic acid with sugar molecules and are also polymers of flavonoids (Goel et al., 2005). It has been reported that condensed tannins may have either harmful or beneficial effects on livestock, depending on their source and concentration in the diet (Makkar, 2003).

In a study conducted by Min et al. (2008), the authors reported that tannins exhibit antimicrobial and bacteriostatic effects. It was also noted that live weight gain in castrated male calves grazing on winter wheat pasture could be improved, while the risks of bloating and methane production were reduced (Min et al., 2006). Furthermore, tannins can decrease the ruminal degradability of proteins, leading to an increase in the amount of protein that reaches the abomasum and small intestine (Teferedegne, 2000). In addition to their bacteriostatic and bactericidal effects in ruminants, tannins can inhibit the enzymatic activities of rumen microorganisms (Faixova and Faix, 2005). It is reported that when the tannin content in the ration exceeds 5% on a dry matter basis, dry matter consumption deteriorates due to deterioration of feed palatability. In addition, tannins disrupt fiber and protein digestion by affecting trypsin and alpha amylase enzymes (Hervas et al., 2003). Condensed tannins, in particular, inhibit the activity of several rumen microorganisms, including biohydrogenating bacteria (Patra and Saxena, 2009). Studies on lactating and beef cattle have shown that including condensed tannins in the diet increases the levels of conjugated linoleic acid (CLA) in both milk and meat (Patra and Saxena, 2011). Vilalba et al. (2002) found that when animals were offered a choice between feeds containing low or high levels of tannins and protein, they preferentially selected the high-protein feeds. Additionally, tannins bind to proteins in the rumen, reducing nitrogen availability, which may negatively affect microbial growth. Therefore, incorporating high-protein diets could yield positive results. On the other hand, animals consuming toxic substances experience an increased demand for macronutrients such as energy and protein to support more effective detoxification (Pfister et al., 2012).

Therefore, the present study was carried out to determine the effects of diet protein and tannin contents on the growth performance of lambs.

Materials and Methods

Animal, feed material and experimental design

A total of 36 male Çukurova meat-type lambs (3/8 Rambouillet, 3/8 Ile de France, 1/8 Ivesi, 1/8 Chios) with an average live weight of 24.69 ± 0.87 kg, obtained from the Sheep Husbandry Unit at the Research Center of Çukurova University in the Faculty of Agriculture, were used in the study. For the experiment, four experimental groups of nine lambs each were formed. Each group of nine lambs was further divided into three sub-groups, each consisting of three lambs.

The diets with varying levels of protein and tannin were formulated isocalorically (Table 1). Oak tannin extracted from acorn served as the source of tannin, and its content is detailed in Table 2. The total mixed ration (TMR) used in the study consisted of 10% chopped alfalfa hay and 90% concentrate feed.

The feedstuff and nutrient contents of the concentrate feeds are presented in Table 1. The metabolizable energy of the experimental diet was calculated according to TSE 9610 standarts. Throughout the study, the lambs were provided with ad libitum access to feed and fresh water.

Data and sample collection

The feed consumption was measured on a group basis, while the live weights were measured individually and weekly. Feed conversion ratio was calculated as feed intake/live weight gain. At the end of the experiment, rumen fluid samples were collected from the lambs using a rumen drencher. The concentrations of acetic acid, isobutyric acid, butyric acid, propionic acid, isovaleric acid, and valeric acid were determined using the method described by Wiedmeier et al. (1987).

The total gas and methane production of the experimental treatments under in vitro conditions were measured using the gas production technique outlined by Menke and Steingass (1988).

Table 1. Composition feedstuff and nutrient contents of concentrate feeds used in the study

Crude Protein		**14**		**18**	
Tannin (%)		**0**	**4**	**0**	**4**
Feedstuffs (%)					
Barley		48.70	43.50	47.50	46.80
Cottonseed		16.10	8.20	6.10	0.00
Corn		10.00	10.00	10.00	10.00
Sunflower seed		9.70	12.60	29.50	32.70
Wheat bran		9.00	15.20	0.40	0.10
Marble powder		1.90	1.90	1.90	1.70
Salt		0.50	0.50	0.50	0.50
Molasses		4.00	4.00	4.00	4.00
Vitamin-		0.10	0.10	0.10	0.10
Dicalcium		0.00	0.00	0.00	0.10
Tannin		0.00	4.00	0.00	4.00
TOTAL		**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrient Content					
Dry	%	88.82	88.70	88.68	88.73
ME	Kcal/k	2400.0	2400.0	2400.0	2400.0
Crude	%	14.00	14.00	18.00	18.00
Crude	%	6.49	6.49	6.61	6.54
Crude	%	11.14	9.42	10.58	9.01
Ether	%	2.77	2.43	1.85	1.45
ADF	%	10.68	10.14	12.06	11.43
NDF	%	23.84	23.24	23.16	21.64
RUP	%CP	27.25	25.65	24.78	23.95
NFC	%	41.72	42.53	39.06	41.10

* Every 1 kg of vitamin mineral premix contains; 3,000,000 IU Vitamin A, 600,000 IU Vitamin D3, 6,000 mg Vitamin E, 196,800 mg Magnesium oxide, 10,000 mg Manganese, 10,000 mg Zinc, 10,000 mg Iron, 2,000 mg Copper, 30 mg Cobalt, 160 mg Iodine, 30 mg Selenium, 200,000 mg Toxin Binder.

Table 2. Composition of oak tannin (%)

Parameters	**%**
Dry matter	93.00
Ash	5.06
Crude Protein (CP)	1.81
Water Soluble sugar	17.30
Hydrolyzed Tannin	64.57
In vitro gas test ME, Kcal/kg	1550.00

Statistical analysis

The experiment was conducted in a factorial arrangement with two levels of protein (14% and

18%) and two levels of tannin (0% and 4%), tested using a complately randomized design. Duncan's multiple comparison test was employed to compare the means of the treatment combinations. Data from the experiment were analyzed using the SPSS (2003) software package.

Results and Discussion

Performance

The performance results obtained from the study are presented in Table 3. At the end of the fattening period, live weight gain and feed conversion were not significantly affected by the experimental factors. However, feed intake was significantly (P<0.01) influenced by crude protein levels, as well as by the interaction between crude protein and tannin levels. Lambs receiving the higher protein diet consumed more feed. In the case of low-protein diets, the inclusion of tannin decreased feed intake, while tannin inclusion in high-protein diets resulted in increased feed intake.

Rumen volatile fatty acids and in vitro gas production

The concentrations of acetic and propionic acid, two major rumen volatile fatty acids, were unaffected by both the main effects (protein and tannin) and their interaction (P>0.05).

The levels of butyric and valeric acid in the rumen fluid were significantly affected by tannin and the interaction between tannin and protein level (P<0.05). Specifically, the inclusion of tannin decreased the levels of butyric acid (16.86 vs. 9.83 mmol/L) and valeric acid (1.10 vs. 0.75 mmol/L) in the rumen fluid.

Both protein level and tannin level had a significant effect on the concentration of isobutyric acid (P<0.05). Increasing the protein content in the diet led to an increase in isobutyric acid, whereas the inclusion of tannin resulted in a decrease in its concentration. The level of isobutyric acid in the rumen fluid decreased progressively in lambs fed low-protein diets with tannin, with a more pronounced decrease in lambs on high-protein diets.

The concentration of isovaleric acid was only significantly affected by the protein content of the diet (P<0.05), with higher protein levels leading to increased isovaleric acid concentrations (0.32 vs. 0.67 mmol/L).

Protein level had a significant effect on both 12-hour total gas and 24-hour methane production. Increasing the protein level in the diet decreased total gas production at 12 hours but increased methane production at 24 hours.

Tannin levels also affected gas production. The inclusion of tannin in the diet increased both 6-hour gas production and 24-hour methane production. However, this effect was dependent on the protein content of the diet. In low-protein diets, the inclusion of tannin decreased gas production, while in high-protein diets, tannin inclusion increased gas production values.

The interaction between protein and tannin levels significantly influenced all gas production values (P<0.05). Specifically, while the inclusion of tannin in the low-protein diet decreased both total gas and methane production after 24 hours, the inclusion of tannin in the high-protein diet resulted in increased gas and methane production.

In the current study, live weight gain and feed efficiency were not affected by the factors, while feed consumption was influenced by the dietary protein level and the interaction between tannin and protein levels. High-protein diets increased feed consumption, whereas the use of tannin in the low-protein diet reduced feed consumption, while it increased feed consumption at high protein levels. As reported by Thomas et al. (2007) on Merino lambs, increasing dietary protein levels would linearly increase dry matter intake. Similarly, Titi et al. (2000) and Haddad et al. (2001) found that a dietary protein level of 16% crude protein (CP) is the optimum for proper dry matter intake, daily weight gain, and feeding efficiency in Awassi sheep. A ruminal ammonia concentration lower than 5 mg/dL may reduce

Table 3. Performance, rumen volatile fatty acids and in vitro gas production findings

Crude Protein (CP, %)	14		18		SEM	P		
Tannin (T, %)	0	4	0	4		CP	T	CP X T
0-8. Week								
LWG, g/day	246.35	253.45	285.54	263.59	16.72	0.15	0.66	0.39
FI, g/day	1657.30^{b}	1563.85^{b}	1653.61^{b}	1778.29^{a}	33.15	0.00	0.64	0.00
FCR	7.36	6.44	5.96	6.85	0.60	0.42	0.98	0.14
Rumen Volatile Fatty Acid Profile (mmol(L)								
Acetic	65.93	64.53	73.26	60.11	5.14	0.78	0.17	0.27
Propionic	26.91	24.90	26.08	26.47	2.49	0.88	0.75	0.63
Butyric	15.11^{b}	10.57^{c}	18.60^{c}	9.09^{a}	0.87	0.26	0.00	0.01
Valeric	1.01^{a}	0.99^{a}	1.18^{a}	0.51^{b}	0.13	0.25	0.02	0.02
Isobutyric	0.64^{b}	0.29^{c}	1.20^{a}	0.37^{bc}	0.10	0.00	0.00	0.02
Isovaleric	0.33^{b}	0.31^{b}	0.76^{a}	0.58^{ab}	0.11	0.00	0.39	0.47
In vitro gas production data (ml)								
Total gas, 6 hours	26.25^{b}	25.25^{b}	24.25^{b}	28.75^{a}	0.66	0.28	0.02	0.00
Total gas, 12 hours	39.25^{a}	36.50^{b}	34.00^{c}	38.00^{ab}	0.62	0.01	0.33	0.00
Total gas, 24 hours	59.75^{a}	51.75^{b}	48.25^{b}	60.25^{a}	1.23	0.25	0.13	0.00
Total methane, 24 hours (%)	17.88^{c}	18.30^{c}	19.88^{b}	21.93^{a}	0.26	0.00	0.00	0.01

^{a,b} Significant of interaction effect on group averages indicated by different letters ($P < 0.05$).

LWG: Live weight gain, FI: Feed intake, FCR: Feed conversion ratio

microbial growth and performance in ruminants (Satter and Roffler, 1975). The high protein levels in the present study may supply sufficient ruminal ammonia-nitrogen to enhance nutrient digestibility and encourage feed intake. It is generally accepted that the presence of tannin in the ration limits dry matter intake. However, it is also stated that the use of tannin up to 5% in the ration does not affect feed intake (Besharati et al., 2022) or that tannin content does not change dry matter intake in sheep (Cabral Filho et al., 2013). In addition, it is stated that some animals fed with high tannin content feeds adapt to this situation by increasing the amount of proline in their saliva (Butter et al., 1999). In the current study, the increase in dry matter intake along with the increase in tannin and protein levels may be attributed to the effect of breed or to the increased nutrient requirement for detoxification of tannin as stated by Pfister et al. (2012).

Hristov et al. (2004) investigated the effects of dietary protein level and protein degradability on rumen fermentation and found that the ratio of acetate to propionate in rumen fluid was not affected by protein level. However, they observed that the concentrations of isovalerate and isobutyrate increased as protein levels rose, similar to the findings in the present study. Similarly, Broderick (2003) reported that high protein intake led to an increase in branched-chain fatty acids in the rumen as the protein content of the diet increased. As noted earlier, condensed tannins prevent rumen protein degradation by binding to protein in the rumen. Isoacids are primarily produced by the degradation of amino acids in the rumen, and it is well established that tannins reduce protein degradation in the rumen (Teferedegne, 2000; Vilalba et al., 2002). Therefore, it is expected that the levels of isoacids, which are products of protein degradation, would be influenced by tannin levels at different protein concentrations, particularly in high-protein diets.

The interaction between protein and tannin levels significantly affected all gas production values in this study. While total gas production and total methane production decreased after 24 h in lambs receiving tannin supplementation with a low protein diet, both values increased in lambs receiving high protein tannin. Essentially, tannins are reported to reduce methanogenesis by suppressing the activity or population of methanogenic bacteria or by reducing the breakdown of feeds in the rumen and preventing hydrogen release (Tavendale et al., 2005). Similarly, it is well known that they have a high affinity for proteins, especially those rich in proline (Perez-Maldonado et al., 1995), and that this leads to the formation of tannin-protein complexes. However, in this study, the high amount of protein taken with the feed or the fact that the tannin amount in the diet was lower than 5% given in the literature may explain the current situation by reducing the formation of this complex. It has also been stated by different researchers that the use of low levels of different tannin sources in the diet does not affect methanogenesis (Sliwinski et al., 2002; Beauchemin et al., 2007).

Conclusion

In conclusion, increasing the protein level in the diet and/or incorporating tannin into the diets of meat lambs starting fattening at 25 kg did not significantly affect fattening performance. However, when considering isoacid concentrations as an indicator of rumen protein degradation, it can be inferred that protein degradation was reduced by the inclusion of tannin in the diet.

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