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THE EFFECT OF SUPPLEMENTATION OF OAK TANNIN EXTRACT ON DIGESTIBILITY, METABOLISABLE ENERGY, METHANE PRODUCTION AND AMMONIA PRODUCTION IN LAMB DIETS

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Abstract: The aim of current experiment was to determine the effect of supplementation of oak tannin extract on gas production, methane production, digestibility, metabolisable energy and ammonia production of lamb diets using *in vitro* gas production technique. Oak tannin extract was included into total mixed ration at the 0, 2, 4 and 6 % on a dry matter basis. Although supplementation of oak tannin had no significant effect on gas, methane whereas supplementation had a significant effect on ammonia production of lamb diets. Gas and methane production of total mixed rations ranged from 50.25 to 53.25 ml and 7.72 to 8.15 ml respectively. Ammonia concentration of mixed rations ranged from 54.97 to 62.67 mg/100 ml. The decrease in ammonia of lamb diets per g oak supplementation was 0.1263 mg /100 ml. Metabolisable energy and organic matter digestibility of lamb diets ranged from 10.42 to 10.80 MJ kg DM and 70.27 to 73.02 % respectively. This study clearly showed that oak tannin had an anti-proteolytic potential for ruminant animals and supplementation of oak tannin significantly reduced ammonia production without compromising digestibility of diets. Therefore, oak tannin can be used to manipulate the rate and extent of degradation of protein in the rumen. However, before large implication, oak tannin should be further investigated using in vivo experiment to determine the toxic level of oak tannin in ruminant animals.

Keywords: Oak tannin, Methane production, Ammonia production, Total mixed ration

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1. Introduction

Methane and NH_3, which are produced when protein and carbohydrates ferment in the rumen, pose a threat to the environment and deplete dietary energy and nitrogen that could be used to produce milk and meat (FAO, 2006; Eckard et al., 2010; Ozkan, 2016). Methane is created by Archaea from the metabolic H_2 that rumen microbioata produce (Demeyer and Van Nevel, 1975; McAllister and Newbold, 2008). Amino acid deamination and feed protein degradation are caused by hyperammonia-producing bacteria. Nitrous oxide emissions from the rumen's extensive protein and amino acid breakdown produce a significant amount of NH_3 and urea, which may be expelled with urine and contribute to ground water contamination and greenhouse gas emissions (Weimer, 1998).

Significant attention has been paid to tannin, essential oils and saponin, which are derived from plants with antibacterial, antifungal, and antioxidant qualities to manipulate rumen fermentation since the use of antibiotics as growth promoters was outlawed (Cowan, 1999; Waghorn et al., 2002; Kamalak et al., 2005; Kamra et al., 2006; Castillejos et al. 2006; Benchaar et al., 2007; Garcia et al., 2007; Ozkan et al., 2016).

Numerous studies have indicated that dietary tannins may be able to reduce the amount of methane produced in the rumen (Woodward et al., 2001; Waghorn et al., 2002; Kamra et al., 2006; Ozkan, 2016). Tannin blocks the production of methanogens directly or indirectly by inhibiting protozoa. (Animut et al., 2008; Bhatta et al., 2009; Jayanegara et al., 2009). By using tannins at low concentrations, it may be possible to boost the efficiency of microbial protein synthesis and reduce rumen protein breakdown (Makkar, 2000). However, there is limited information about the effect of oak tannin on the fermentation parameters of lamb diets. It was hypothesized that the oak tannin may decrease the methane production and extensive degradation of protein in the rumen.

The aim of current experiment was to determine the effect of supplementation of oak tannin extract on gas production, methane production, digestibility, metabolisable energy, and ammonia production of lamb diets using *in vitro* gas production technique.

2. Materials and Methods

Iso-caloric and iso-nitrogenic lamb diets formulated using the concentrate ingredients namely barley grain, soybean meal and alfalfa hay is given in Table 1. Oak tannin extract was included into lamb diets at the 0, 2, 4 and 6 % on a dry matter (DM) basis.

In vitro gas production experiment was carried out with permission of Animal Ethic committee of Kahramanmaraş Sütçü Imam University, Faculty of Agriculture (Protocol No: 2021/03-2). *In vitro* gas production of lamb diets was determined for 24 h (Menke et al., 1979). Rumen fluid was obtained from three fistula sheep fed with a diet containing alfalfa hay and concentrate before morning feeding. The rumen fluid was transferred into laboratory and combined with buffer solution. The samples were incubated in triplicate in glass syringes with buffered rumen fluid for 24 h in the water bath set at 39 °C.

The ME and OMD of diets were estimated using the equations 1 and 2 as follows (Menke and Steingass, 1988).

$$ME\ (MJ/kg\ DM) = 1.68 + 0.1418*GP + 0.073*CP + 0.217*EE - 0.028*CA \quad (1)$$

$$OMD\ (\%) = 14.88 + 0.889*GP + 0.45*CP + 0.651*CA \quad (2)$$

here, GP = Gas production for 24 h (ml), CP = Crude protein (%), EE: Ether extract (%), CA: Crude ash (%)

Methane content (equation 3) of gas produced was determined using the infrared methane analyzer (Goel et al., 2008).

$$\text{Methane production (ml)} = \text{Total gas production (ml)} \times \text{Percentage of methane (\%)} \quad (3)$$

After determination of gas production, the content of syringes was transferred into glass bottles for distillation unit to determine ammonia-N content of lamb diets. The Ammonia-N content of diets were calculated as given in equation 4.

$$mg\ N\ (NH3\text{-}N) = 0.1 \times 14 \times (A-B) \quad (4)$$

2.1. Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine the effect of oak tannin on *in vitro* gas production, methane production, ammonia production, metabolisable energy and organic matter digestibility of lamb diets. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $P<0.05$.

3. Results and Discussion

The effect of oak tannin on *in vitro* gas production, methane production, ammonia production, metabolisable energy and organic matter digestibility of lamb diets is given in Table 2. Supplementation of oak tannin had no significant effect on gas production, methane production, metabolisable energy and organic matter digestibility of lamb diets. Gas production and methane production ranged from 50.25 to 53.23 ml and 7.447 to 8.15 ml respectively. The percentage of methane gas ranged from 14.90 to 15.32%.

It was suggested that tannin might reduce the methane production through reduction in fibre digestion and inhibition of growth of methanogens (Tavendale et al., 2005). Previous investigation showed that the supplementation of tannin from different sources to diets had a significant effect on gas production and methane production (Jayanegara et al., 2015) whereas supplementation of tannin had no significant reduction in methane production in the current experiment. The differences between these studies are likely to be associated with difference in tannins used (Jayanegara et al., 2015).

Metabolisable energy and organic matter digestibility of lamb diets ranged from 10.42 to 10.80 MJ kg DM and 70.27 to 73.02 % respectively.

Oak tannin supplementation significantly decreased the ammonia production of lamb diets. Ammonia concentration of mixed rations ranged from 54.97 to 62.674 mg/100 ml. This result obtained in the current study is in agreement with findings of Pinski et al. (2015) who found that condensed tannin decreased ammonia production.

Table 1. The chemical composition of diets including oak tannin (%DM)

	Diets			
Ingredients	0%	2%	4%	6%
Barley	600	600	600	600
Oak tannin	0	20	40	60
Oil	1	2	3	4
Soybean meal	109	118	130	142
Alfalfa hay	264	234	201	168
NaCl	10	10	10	10
CaCO3	15	15	15	15
Min-Vit	1	1	1	1
Total	1000	1000	1000	1000
ME (Kcal)	2654	2652	2653	2654
CP	170	170	170	170

CP= crude protein, ME= metabolisable energy.

Table 2. The effect of oak tannin on *in vitro* gas production, methane production, ammonia production, metabolisable energy and organic matter digestibility of lamb diets

	Diets					
Parameters	0	20	40	60	SEM	Sig.
Gas	50.75	51.25	53.25	50.25	1.567	N.S
CH_4(%)	15.22	15.00	15.32	14.90	0.343	N.S
CH_4(ml)	7.72	7.70	8.15	7.47	0.272	N.S
NH_3(mg/100 ml)	62.67^{a}	59.00^{ab}	56.85^{b}	54.97^{b}	1.389	***
ME (MJ)	10.42	10.52	10.80	10.40	1.389	N.S
OMD (%)	70.97	71.32	73.02	70.27	1.388	N.S

^{ab} same common superscripts letters in the row shows statistical similarity (P>0.05), SEM= standard error mean, Sig= significance level, ***= P<0.001.

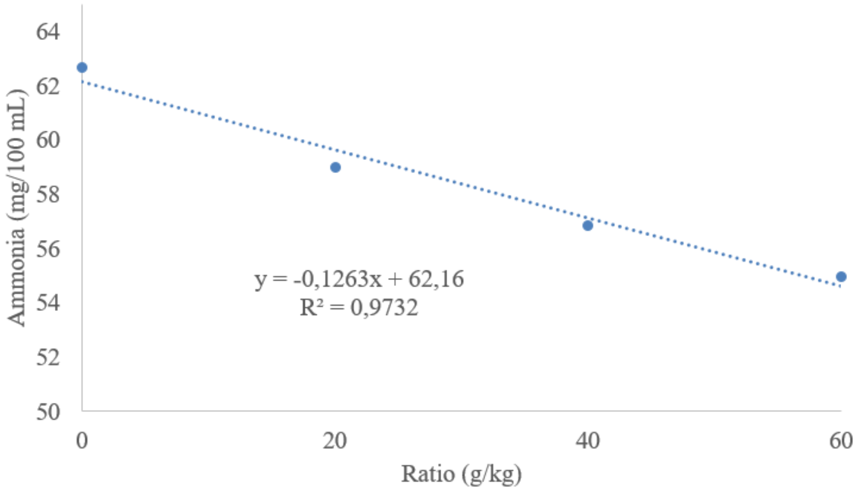

Figure 1. The relationship between oak tannin and ammonia production.

The relationships between oak tannin doses and ammonia production is given in Figure 1. Ammonia production decreased with increasing level of condensed tannin. The decrease in ammonia of lamb diets per g oak supplementation was 0.1263 mg /100 mL.

The clearly showed that supplementation of oak tannin had a significant effect on the degradation of protein in lamb diets without compromising digestibility of diets. Therefore, it can be suggested that oak tannin can be included into lamb diets to prevent the extensive degradation of protein of diets. However, care must be taken into consideration that hydrolysable tannin can be toxic to ruminant animals (Jayanegara et al., 2015). The safe level of oak tannin inclusion should be determined before large implication.

4. Conclusion

This study clearly showed that oak tannin had an anti-proteolytic potential for ruminant animals and supplementation of oak tannin significantly reduced ammonia production without compromising digestibility of diets. Therefore, oak tannin can be used to manipulate the rate and extent of degradation of protein in the rumen. However, before large implication, oak tannin should be further investigated using *in vivo* experiment to determine the toxic level of oak tannin in ruminant animals.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	A.S.D.	A.K
C	50	50
D	50	50
S	50	50
DCP	50	50
DAI	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

In vitro gas production experiment was carried out with permission of Animal Ethic committee of Kahramanmaras Sutcu Imam University, Faculty of Agriculture (approval date: March 02, 2021, protocol code: 2021/03-2).

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