

THE EFFECT OF POMEGRANATE PEEL AND PISTACHIO HULLS ON PERFORMANCE AND ENTERIC METHANE EMISSIONS IN STRAW-FED LAMBS (*Ovis aries* L.)

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(Received 10th Sep 2018; accepted 29th Nov 2018)

Abstract. This trial was conducted to determine the effect of pomegranate peel (PP) and pistachio hulls (PH) added to wheat straw (WS) on some performance parameters and production of some greenhouse gases in female Awassi lambs (*Ovis aries* L.). This research was conducted in Sanliurfa province in Southeastern Turkey and it was done at Harran University Faculty of Agriculture. Fifteen lambs were used in a completely randomized design with 52 days periods (45 days of fattening performance and 7 days of gas measurements) and three treatments: control (90% concentrate + 10% WS); PP (90% concentrate + 5% PP + 5% WS); and PH (90% concentrate + 5% PH + 5% WS). Emissions of nitrous oxide (N₂O) and enteric methane (CH₄), and carbon dioxide (CO₂) production were also measured during the last week (7 days). Performance parameters did not change with inclusion of PP and PH in the diet for 0–6 weeks. However, inclusion of PP decreased feed intake during 0–3 weeks. The CO₂ emissions (as g day⁻¹ lamb⁻¹ and g kg⁻¹ dry matter intake) significantly increased in the PP and PH groups, but CH₄ emissions did not significantly change. Total N₂O (g day⁻¹ lamb) emissions from feces were affected by inclusion of PH in the diet. The PH supplementation showed a strong tendency for reducing N₂O emissions from feces of lambs.

Keywords: methane, by-product, manure, sheep, tannin

Introduction

The increase in individual consumption causes the amount of vegetable waste to increase rapidly. An important method of recovery in sustainable vegetable waste management is composting. Another important option is to evaluate wastes such as fruit peel as animal feed. Production of pomegranate and pistachio has increased due to the South Anatolian Project in the Southeastern Anatolia region of Turkey. According to the Turkish Statistics Institution (TUIK, 2016), 144 000 tonn of pistachios were produced in Turkey during 2015. Of this, 30% is produced in Sanliurfa Province, with an average for the last 5 years of 38000–42000 tonn/year (TUIK, 2015). In Sanliurfa, 7 tonnes of soft shells or hulls are produced annually. The pistachio soft hull (PH) is red or burgundy colored and is separated by post-harvest crushing. It is composed of fruit stem, leaf, fruit content, and mesocarp (middle shell) (Shakeri et al., 2014). The PH obtained from the factories also contains some tree leaves.

The PH contains high concentrations of non-structural carbohydrate (36%–40%), moderate concentrations of crude protein (11.4%–13%), 6% fat, and 30%–33.3% neutral detergent fiber - NDF (Shakeri et al., 2013). The PH contains high concentrations of polyphenolic compounds (7.8%) and the halves of these compounds are composed of tannins (Shakeri et al., 2013). Therefore PH cannot enter as only one rough feed in ratio. In Sanliurfa, 9600 tonnes of pomegranates are produced per year

(TUIK, 2016). Since 48% of the total weight of pomegranate consists of soft crust, 4800 tonnes of these are produced annually. Tannins, which are found in pomegranate seeds and peels (PP) or PH, exhibit inhibitory effects on protein binding and bacterial proliferation and have antimicrobial, antioxidant, and anti-inflammatory effects in digestion both *in vivo* and *in vitro* (Adams et al., 2006; Jayaprakasha et al., 2006; Zarei, 2013).

N₂O is a dangerous greenhouse gas and expected to increase by 35-60% by 2030 with an increase in demand for meat and dairy products (IPCC, 2007). PP or PH containing tannins may improve N utilization efficiency and thereby decrease the N content of manure, which, in turn, may affect N₂O emissions because less N is available to the denitrifying bacteria that use the manure as substrate.

The addition of saponins from PP can thus modify the C and N contents of sheep manure. Sheep (*Ovis aries* L.) produce 8 kg of enteric methane (CH₄) gas per animal per year (Broucek, 2014) and any CH₄ reduction potential due to tannins suppressing CH₄-producing microorganisms in the rumen will be determined *in vivo*. PP and PH can also provide an advantage especially for lowering the higher fiber concentration that comes with the wheat straw. This study will also investigate the potential of PP and PH as forage sources to reduce the use of wheat straw.

Materials and methods

Animal and feed material

The study was conducted between December and November at Sanliurfa province in the Southeast Anatolian Region of Turkey, which lies on 37°9'32.9364 latitude (N) and 38°47'48.8724 longitude (W). Research on animals was conducted according to the institutional committee on animal use. Ethics committee approval was obtained from DOLLVET animal vaccine production center (DOLLVET-HADYEK-2015/01). The experiment was carried out on 6-month-old female Awassi lambs (*Ovis aries* L.) for 52 days: 45 days of fattening performance and 7 days of gas measurements. The total of 15 lambs was divided into three groups (Table 1) with five individual compartments of similar individual weight (29.13 ± 3.18 kg) and age (Fig. 1).

Table 1. Experimental groups

Groups	Concentrate	Forage
1-Control	90 (%)	10 (%) straw
2-Pomegranate peel (PP)	90 (%)	5 (%) straw + 5 (%) pomegranate peel
3-Pistachio hulls (PH) ¹	90 (%)	5 (%) straw + 3 (%) pistachio hulls + 2 (%) pistachio hulls and leaves

¹; PH in the study consists of a mixture of pure pistachio hull and some leafy fraction

The rations were given to the animals as mixed forage and concentrated feed total mixed ration (TMR; Table 2). The rate of WS used in the control ration was 10%; PP and PH replaced half of the WS to form the other feeding groups. Dry PP containing some crushed grain was obtained by drying in the sun at 40 °C for 1 week.

The PH used in the study consisted of pure soft burgundy fraction and a small amount of pistachio tree leaves. Dry PP and WS (3–5 cm) were crushed; and the PH was used without treatment (Table 3). In order not to stress the lambs, the feeding was

done by adding every morning during the week as a little more than the maximum amount that they could consume daily. At the end of each week, the remaining feed was weighed and subtracted from the feed added for one week and divided into 7 days. Thus, daily feed consumption was found.

Table 2. Contents of total mixed ration (TMR) used in the lamb growth diet in the experiment (g/kg as fed basis)

Feed ingredient	Control	PP ¹	PH
Barley	216.9	216.9	216.9
Corn	266.58	266.58	266.58
Soybean meal	165.78	165.78	165.78
Wheat bran	225	225	225
Limestone	19.44	19.44	19.44
Salt	5.4	5.4	5.4
Vit + min.pre. ²	0.9	0.9	0.9
Wheat straw	100	50	50
PP	0	50	0
PH	0	0	50
Total	1000	1000	1000
Nutrient values (as fed basis)			
Dry matter (%)	89.7	89.4	89.6
ME (Mcal/kg)	2.4	2.4	2.4
Crude protein (%)	16	15.9	15.9
Ether extract (%)	3	3	3
Crude fiber (%)	9.7	9.25	8.4
ADF (%)	11.6	11.8	10.2
NDF (%)	26.8	25.3	24.4
Ash (%)	4.08	4.2	4.1
Calcium (%)	0.87	0.86	0.86
Phosphorus (%)	0.6	0.59	0.59
Sodium (%)	0.31	0.3	0.32
Tannin (mg eq/g) ³	38.25	68.44	79.36
Saponin (mg/g) ³	15.8	54.71	15.6

¹; PP, pomegranate peel; PH, Pistachio hulls. ME, methabolic energy, ²; 1 kg vitamin mineral mixture: 15.000 IU vitamin A, 25 mg Vit E, 4000 IU Vit D3; Trace minerals: Fe: 50 mg, Co: 0.1 mg, Mn: 50 mg, Se: 0.2 mg, I: 0.8 mg, Cu: 10 mg, Zn: 50 mg, ³; Dry matter bases

Table 3. Nutritional values of plant byproducts used in the experiment (as fed basis)

	PP ¹	Pistachio hulls	Pistachio hulls and leaves	Wheat straw
Dry matter (%)	91.00	92.00	92.00	91
Crude ash (%)	8.00	9.00	8.50	8.0
Crude protein (%)	5.83	7.03	4.40	8.2
ADF (%)	48.50	20.19	20.6	52
NDF (%)	55.20	24.39	28.2	80

¹; PP, pomegranate peel; ADF, acid detergent fiber; NDF, neutral detergent fiber

CH₄, carbon dioxide (CO₂) and nitrous oxide (N₂O) measurements in respiration chamber

The enteric CH₄ measurement was initiated in lambs adapted to their feed for 45 days. For this purpose, an isolated container (dimensions 2 × 2 × 2 m) with two chambers was turned into a respiration chamber with controlled air inlets and outlets (Fig. 1).



Figure 1. Individual compartments and respiration chamber

As there was herd/flock of psychology in the lambs, the rooms were arranged with glass windows. The lambs were able to see each other and reduce stress. In addition, they were left in the room for 24 hours in order to reduce the effect of stress and acclimate to the room environment. Two pipes (as inlet and outlet) were each attached to a fan of approximately 0.25 m³/sec. air supply capacity in each room. The pipes were cylindrical chimneys with a diameter of 10 cm and a length of 30 cm and fresh air was pumped into each room with a fan in the inlet pipe and extracted by another fan in the outlet pipe. Gas emissions were measured by subtracting the concentrations of the gases in the air entering and leaving the chamber.

After the lambs were taken to the room, the fans were activated and the temperature and humidity inside and outside the room were recorded. For each animal, air samples were taken with syringes at least 5–6 times for both inlet and outlet from fan cylinders during daylight and were immediately sealed with an airtight cover. After the end of day light, the sampling was continued and samples were taken every 2 hours. During the gas measurement period, the lambs stayed in the respiratory room with feeders for 23 hours from 08.30 h in the morning until 07:30 h the following morning.

At intervals of 1 h, the rooms were ventilated and the stool and urine of the previous lamb were removed. Gases were identified with gas chromatography (GC) on sampled syringe tubes as ppm for entering and exiting air (SRI Instruments-European Greenhouse GC System® - Germany). A 3-m Hayesep D packed column

was used for CO₂, CH₄, and N₂O diagnosis. Operating conditions for GC: injector temperature was 95°C, column temperature was 85°C, and detector temperature was 320°C–350°C.

Gas concentrations were calculated using the following formula (Petrucci et al., 2010) in the incoming and outgoing air (Eq. 1):

$$CO_2, CH_4, N_2O = \left[C \cdot Ma \frac{P}{RT} V \cdot A \right] \quad (\text{Eq.1})$$

CH₄, CO₂ and N₂O in g/sec, C is fresh air entering the room and gas concentration in the leaving air (ppm), *Ma* is gas molecular weight (g/mol), *P* is barometric air pressure (Pa), *V* is entering and leaving air velocity (m/sec), *R* is the universal gas constant (8.31 J/molK¹), *T* is ambient temperature, and *A* is area of a cross-section of the cylindrical chamber (0.0250 m²).

Chemical analyses

Dry matter, crude ash, and crude protein analyses of feeds used in the trials were performed according to the classical AOAC (1998) procedures. Fiber analyses (acid detergent fiber - ADF and NDF) were performed with an ANKOM Fiber Analyzer according to Van Soest et al. (1991).

Tannin analysis

A standard tannin analysis curve was prepared according to Makkar et al. (1995) and used to calculate tannin contents (Table 4). To prepare the standard curve, 2, 4, 6, 8, 10, and 12 µg/ml pure tannin was placed in the test tubes, 500 µl of Folin Ciocalteau separator was added and mixed for 3 min. After addition of 2500 µl of Na₂CO₃, the tubes were shaken for 60 min, and absorbance measured at 725 nm. The regression equation obtained from reading values revealed tannin concentrations of the feed samples.

The feed samples milled to 1 mm were treated with the condensed tannin (CT) solution. For the CT solution, 0.05 g of FeSO₄ was added to the tube and 0.015 g of ground sample was added. Then 2 ml of 0.55 M butanol–HCl reagent was added to each tube and then mixed with a vortex. The test tube was tightly sealed, kept at 97–100 °C for 1 h, and absorbance values measured at 580 nm (Karaogul, 2011).

Table 4. Determination of condensed tannin value from standard curve

Items	Standard compound	Calibration equation	R ²	LOD/LOQ ³ (µg l ⁻¹)	Q ⁴ (mg equivalent g ⁻¹ dry matter)
Wheat straw	Tannic acid	Y = 0.053×+0.0067	0.98	0–12	35.59 ± 0.74
PP ¹	Tannic acid				103.52 ± 1.65
Pistachio pure hulls	Tannic acid				173.96 ± 2.12
Pistachio hull and leaf (PH) ²	Tannic acid				89.05 ± 0.57

¹ ; PP, pomegranate peel, ² ; PH, consists of pure soft burgundy fraction and a fraction containing a quantity of pistachio tree leaves, ³; LOD/LOQ: Limit of detection/Limit of quantification, ⁴; Quantification

Statistical analyses

Performance data were analyzed in the One-Way ANOVA procedure according to the experimental design of the three-group randomized design and the treatment combinations were compared with the Duncan's test using SPSS. As for gas emission values, the data were statistically analyzed with Levene's test and Shapiro Wilk test for equality of variances and the normality assumption, respectively ($P < 0.05$). Then, these data were analyzed by using Kruskal Wallis H test (SPSS, Version 22, 2013) and presented with median and IQR values (interquartile range) in Table 5.

Table 5. Non-parametric results of gas emission values

Groups	Emission (g/day lamb)	Means	Standard deviation	Median	IQR ⁽²⁾	P-value
Control	CH ₄	46.87	26.67	45.26	-	0.21
PP ¹		36.79	13.71	34.06	16.48	
PH ¹		18.72	22.03	11.40	36.36	
Control	CO ₂	514.43	50.9	514.96	412.97	0.04
PP		1079.90	290.6	994.34	924.79	
PH		700.53	91.91	714.25	-	
Control	N ₂ O	186.25	56.84	186.25	-	0.05
PP		182.36	108.42	166.86	206.93	
PH		59.26	21.99	56.25	43.56	

¹; PP, pomegranate peel; PH, pistachio hulls, ²; IQR, interquartile range: quarter value width, the third quadrant and the first quadrant range of an ordered data array

Results

There was no significant difference during the 0-6 week periods of the experiment in terms of the data on the effects of PP and PH additions on feed consumption, live weight change (Fig. 2), and feed conversion ratio (Table 6).

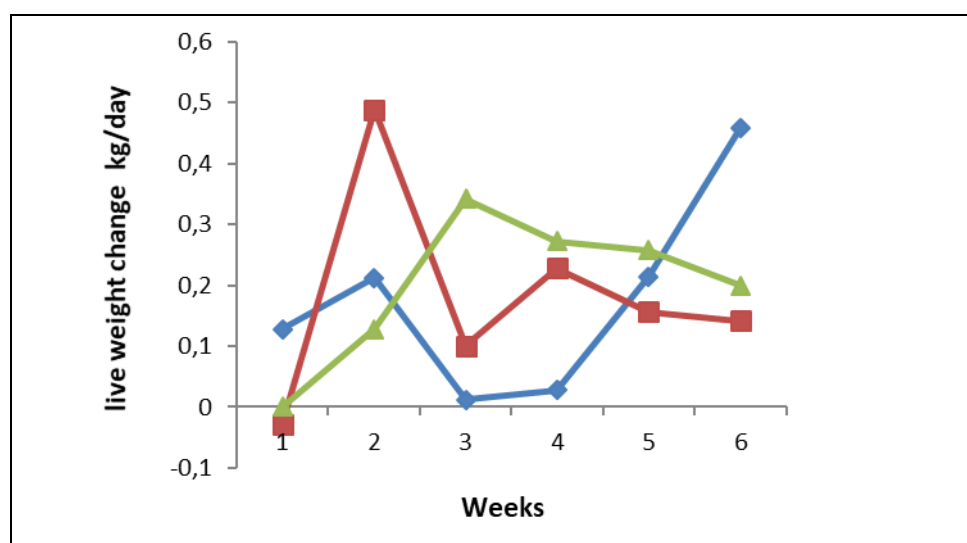


Figure 2. The time dependent changes in live weight gain/change in experimental groups

Table 6. Means of performance data in the experimental groups during the first half and the last half of the trial

	Weeks	Control	PP ¹	PH ²	SEM ³	P-value
Feed consumption (kg/day as fed)	0–3	1.93b ⁴	1.83a	1.93b	0.02	0.02
	3–6	1.90	1.94	1.97	0.03	0.27
	0–6	1.92	1.88	1.95	0.02	0.16
Metabolic energy intake (Mcal/day)	0–3	4.60b	4.41a	4.62b	0.04	0.02
	3–6	4.57	4.62	4.73	0.06	0.25
	0–6	4.61	4.55	4.68	0.05	0.16
Live weight gain (g/day)	0–3	120	220	158	68.8	0.63
	3–6	234	176	244	26.1	0.22
	0–6	213	182	202	29.8	0.81
Feed conversion ratio	0–3	8.19	12.95	15.18	4.59	0.56
	3–6	8.30	13.06	8.40	1.26	0.06
	0–6	9.43	13.9	10.0	1.89	0.37

¹; PP, pomegranate peel, ²; PH, pistachio hulls, ³; SEM, standard error of the mean, ⁴; Different letters show significant differences between the columns in the same row

Except for the first week of the experiment, no significant differences in these performance values were observed. In the first 3 weeks of the experiment ($P < 0.05$), feed consumption was lower in lambs receiving PP (Fig. 3) in terms of trial averages, and similar live weight gains were obtained in all groups ($P > 0.05$).

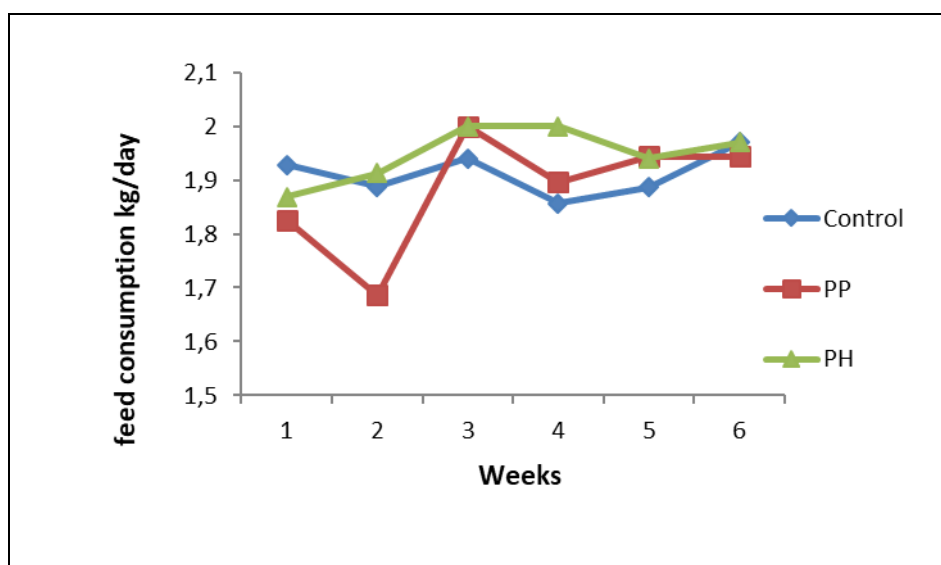


Figure 3. The time dependent changes in feed consumption in experimental groups

Energy consumption was similar throughout the trial in all groups, except during the first weeks ($P < 0.05$). The CH_4 emissions were similar in all groups, but with a decreasing trend for PP and PH groups ($P = 0.07$). However, carbon emissions were higher in the PP and PH groups ($P < 0.05$; Tables 5 and 7). The N_2O emission (g/day lamb¹) in the PH group was lower than in the control and PP group ($P = 0.05$; Fig. 4).

Table 7. Gas emission values according to daily dry matter consumption

	Control	PP ¹	PH ¹	SEM ⁽²⁾	P-value
CH ₄ (g/kg DMI)	24.42	19.74	9.56	5.65	0.07
CO ₂ (g/kg DMI)	268.13a, ³	578.88b	359.31a	70.60	0.03
N ₂ O (g/kg DMI)	100.07b	98.62b	25.97a	34.85	0.03

¹; PP, pomegranate peel; PH, pistachio hulls; DMI, dry matter intake, ²; SEM, standard error of the mean, ³; Different letters show significant differences between the columns in the same row

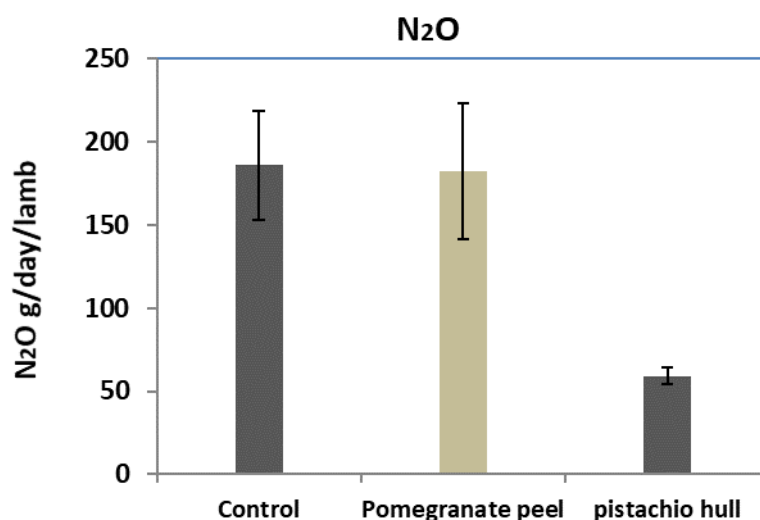


Figure 4. The changes in N₂O emission levels among groups

Discussion

Within the first 3 weeks of the experiment, PP negatively affected feed consumption. This negative effect lasted 3 weeks and became less pronounced during the later weeks of the experiment (*Figs. 2 and 3*) probably due to adaptation to the taste of PP. Provenza (1995) suggests that negative feedback responses diminish over time depending on the negative factor.

The results we obtained were consistent with the results reported by Ghaffari et al. (2014), who feed Afshari and Balouchi male lambs with 30% PH and did not observe any change in feed intake. The CT concentration in some plants did not limit feed consumption at 50 g/kg dry matter (DM) (Barry and McNabb, 1999). However, Bhatta et al. (2002) found that consumption was reduced at CT concentrations above 60 g/kg. In our study, CT at 90.53 g/kg concentrations in the PH groups did not cause a significant decrease in feed consumption except during the first weeks – this may be due to the difference in plant species. Consistent with our findings, Addisu et al (2016) reported that low or moderate concentrations of CT (20–45 mg equivalent/g DM) in feed stuffs did not affect feed consumption. According to Villalba and Provenza (2000), feed consumption of lambs can improve if a low-quality feed is associated with a nutrient. In our study, TMR was preferred in the first 3 weeks in PH groups due to

higher protein concentration of PH than PP. However, it is also possible that the higher saponin content in PP groups may cause fluctuations in feed consumption.

The tendency to similar live weight gain in all groups can be attributed to an internal mechanism related to lambs, but the inclusion of PP as half of the forage had a clear negative effect on the lambs. Abarghuei et al. (2013) suggest that PP contains high concentrations of saponin which reduces protein digestibility due to negative effects on digestion, and decreases feed consumption by reducing the palatability. Compared to PH, the lambs reacted more negatively to the PP, indicating that feeds such as pomegranate are more problematic in terms of taste. Kotsampasi et al. (2014) stated that the addition of PP to the TMR at concentrations of 0, 120, and 240 g/kg did not significantly affect live weight, live weight gain, DM consumption, and feed utilization. Norouzian and Ghiasi (2012) found that PH substituted for up to 30% of forage did not significantly change the performance values of lambs, showed no adverse effects, and could be used a part of forage in lamb fattening. However, PP was tolerated by lambs in later periods of the current study.

The consumption values obtained in our study showed that the CT content of PH was less unappealing for the lambs. According to Sadq et al. (2016), CT has a less reducing effect on digestion and voluntary feed intake. When considering feed consumption, lambs need some time to become accustomed to PH and PP feed. Shabtay et al. (2008) found that fresh PH linearly increased feed consumption and was accompanied by an increased live weight gain – the introduction of tannin-rich PH up to 20% of the ration had positive effects on fattening performance with no harmful effect.

In our study, addition of PP and PH did not significantly affect emissions of enteric CH₄ but there was a declining trend for PP and PH groups in CH₄ emission values when calculated according to kg feed intake ($P = 0.07$). The CH₄ was of enteric origin and CO₂ both enteric and respiratory, because feces in an open environment are not an important source of CH₄ and CO₂. Because of wheat straw's low digestibility, enteric CH₄ production is an important problem but no significant effect of CT in wheat straw-based feeding was observed in our study, and the effects of CT on rumen CH₄ emissions were not clear. The CT generally form complex bonds with ruminal cellulose and reduce hydrogen utilization for methanogenesis (Pinerio-Vazquez et al., 2015).

It is likely that a reduction in WS concentration, the source of cellulose, in the PP and PH groups may have caused the current effect to be hidden. The average daily CH₄ production per animal was 33.5 g over all groups, corresponding to annual CH₄ emission of 12.2 kg per animal. Broucek (2014) found that milk sheep produce 8.4 kg of CH₄ per animal annually, which is lower than that obtained in our study. The difference can be attributed to different breeds used between the studies.

The CO₂ detected may be of both enteric and respiratory origin. The CT can also increase the content of glycoprotein in saliva and stimulate saliva production (Gxasheka et al., 2015). This result shows that high saponin and CT feeds must be chewed more – thus, more O₂ consumption and CO₂ production may have occurred in the PP and PH groups. El Meccawi et al. (2008) found that alfalfa of higher CT content was more effective than *Acacia* in producing body heat. Heat production is an important trigger of high O₂ consumption and CO₂ production in the body.

The N₂O was not of enteric but fecal origin and may be related to possible accumulation of feces in the environment. Every animal kept in the room for 24 h was left with its own external feces. According to this, daily N₂O release per sheep tended to be lower ($P = 0.05$) in the group receiving PH (Fig. 4). Addition of PP and PH

significantly decreased daily N₂O production for kg feed consumption. In this study, CO₂ emission increased in PP and PH groups but decreased N₂O. The global warming potential of N₂O is 298 times stronger than CO₂ and is a greenhouse gas that emerges in animal activities (IPCC, 2007).

The results were promising in terms of nitrogen-based gases. The N₂O production tended to decrease in the PH groups per sheep (*Table 5*) and we had strong indications that N₂O release was related to protein metabolism associated with CT in the rumen and the intestine. We speculate that the lower emissions were due to the lower N content of the feces as a result of greater N binding and inhibition of N transformation. According to Bunglavan and Dutta (2013), 2%–3% CT in the diet may reduce ruminal protein degradation and result in degradation of the tannin–protein complex in the abomasum, and its lower pH (<3.5) may increase the absorption of amino acids in the intestine.

The CT concentration was up to 1% in the PP and PH groups in our study. The results suggested that CT in PH might affect fecal composition by binding proteins in the rumen and so improving protein digestion in the small intestine. The lower emissions from PH were also probably due to the lower contents of mineral N in the manure/feces. An important source of N₂O in feces is ammonia-based nitrogen and it is known that CT decreases ruminal protein dissolution and decreases ammonia concentration. This increases the non-ammonia nitrogen flow in the small intestine (Choi et al., 2012; Naumann et al., 2017).

Conclusion

We found that the use of CT-containing PP and PH feed as half of the wheat straw as roughage feed (WS was 10% in the current study) was a good choice for lamb fattening and production. The CO₂ emissions significantly increased in the PP and PH groups, but they did not effectively reduce the production of enteric CH₄ in the rumen and energy loss through CH₄ for the WS-based feed.

There is significant potential for PH in the fecal emission of nitrogen-based gases. We speculate that the lower emissions were due to the lower N content of the manure as a result of greater N binding and inhibition of N transformation. Thus, some by-products of the food industry have a potential as animal feeds that can reduce N₂O emissions. Reducing the emissions of GHG from ruminants can put such by-products to better use and help in mitigating the adverse impacts of climate change at the same time.

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