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Effects of extracts derived from pistachio by-products on ruminal fermentation and methane production

P. Shakeri[1*], A. Madahian[2] and A. Mokhtarpour[3]

[1]Animal Science Research Department, Kerman Agricultural and Natural Resource Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Kerman, Iran.

[2]Department of Agriculture, Payame Noor University, Iran.

[3]Research Center of Special Domestic Animals, University of Zabol, Zabol, Iran.

*Corresponding author, E-mail address: pirouz_shakeri@yahoo.co.uk

Abstract The objective of this study was to evaluate the effects of different doses of pistachio by-product (PBP) extracts supplemented with alfalfa hay (AH) or barley grain (BG) on microbial fermentation in an *in vitro* fermentation system. The total extracted phenolic compounds were 36.96, 65.78, 67.02 and 8.85% and total extracted tannin contents were 37.11, 59.64, 56.87 and 7.55% when PBP was extracted with water, ethanol 70%, methanol 80% and a mixture of chloroform and methanol, respectively. Adding the mixture of chloroform and methanol extracts to both substrates reduced the gas production ($P<0.01$). Most of PBP extracts caused a significant reduction ($P<0.01$) in ruminal ammonia compared to controls with both substrates while, the effects of PBP extracts on ammonia production were greater for BG than AH based substrate. The concentrations of volatile fatty acids (VFAs) were higher ($P<0.01$) in all solvent extracts compared to control (except the low level of methanol PBP extract) with both substrates. Furthermore, in both substrates there was no decrease in methane productions with the addition of all extracts compared to controls. In conclusion, some of PBP extracts may have a favourable effect on rumen fermentation parameters such as gas production, ruminal ammonia and VFAs concentrations and they can also assist in developing novel feed additives for decreasing the nitrogen excretion in the ruminants.

Keywords: phenolic compounds, tannin, ammonia, VFAs, *in vitro*

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Introduction

Pistachio by-product (PBP) is obtained during de-hulling of fresh pistachio after harvesting and consists of soft external hull, twigs, leaves, kernel and bony shells. Iran is the first producer of pistachio in the world followed by USA and Turkey (FAO, 2015). It has been estimated that PBP production in Iran based on fresh weight is over 500,000 ton annually (Ghaffari et al., 2014a). Using PBP as an animal feed not only decreases forage shortage but also reduces the risk of polluting the environment (Mokhtarpour et al., 2012). During the last few years, several studies have been reported successful inclusion of PBP in the diets of sheep, dairy goat, and dairy cows (Soltani Nezhad et al., 2016; Ghaffari et al., 2014a,b; Mokhtarpour et al., 2012).

The phenolic compounds (PC) and total tannins (TT) of sun-dried PBP have been reported to be 104± 25.6 and 61.6± 21.8 g/kg of DM respectively (Shakeri et al., 2016). Tannins can modify microbial populations, con sequently altering variables such as nutrient digestibility, ruminal ammonia concentrations, methane emissions and VFAs profiles with potential effects on animal metabolism (Al-Dobaib, 2009; Krueger et al., 2010). On the other hand, extraction of secondary metabolites is carried out through costly solvents, such as methanol, ethanol or acetone (Makkar, 2003). However, water has effectively been used as a solvent during extraction to decrease the costs and make PBP extract concentrated and economically feasible treatment for animal diets (Jolazadeh et al., 2015; Mokhtarpour et al., 2016).

Also, PBP extract has a wide range and high concentration of total PC such as anacardic acids (3198 mg/100 g), fatty acids (1500 mg/100 g), and phytosterols (192 mg/100 g) as major components. Carotenoids (4.93 mg/100 g), chlorophylls (10.27 mg/100 g), tocopherols (8.83 mg/100 g), and three triterpene acids (mangiferolic, isomangiferolic and mangiferonic acids) (Grace et al. 2016), which have been shown to possess antimicrobial, antioxidant, anti-inflammatory and antimutage-

nicity properties (Rajaei et al., 2010; Grace et al., 2016).

Therefore, we hypothesize that inclusion of PBP extract to the diet of animals would improve protein metabolism and reduce ruminal ammonia concentrations. Hence, this experiment was conducted to evaluate the effects of different doses of PBP extracts on fermentability and its potential to reduce ruminal ammonia and methane emission with fibrous (alfalfa hay) or concentrate-based (barley grain) substrates.

Materials and methods

Chemical composition

The chemical composition of PBP, alfalfa hay and barley grain samples were analyzed in triplicate for dry matter (DM), crude protein (CP) and ash (AOAC, 2000). CP was estimated by measuring N content using an automatic Kjeldahl analyzer *(*Kjeldahl Vap50 Gerhardt, Germany). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using Fibre Analyser (Fibretec 2010, Auto fiber analysis system (Foss Analytical, Denmark) according to Van Soest et al. (1991) and AOAC (2000), respectively. The concentration of total PC and TT in PBP and its extracts were determined by using the Folin-Ciocalteu reagent with tannic acid as the chemical standard (Makkar, 2003).

Extraction of PBP

The crude extracts were obtained from sun-dried and ground PBP using different solvents, i.e., ethanol (70 ml ethanol/30 ml water), methanol (80 ml methanol/20 ml water), chloroform (50 ml chloroform/50 ml methanol) and water according to Billo et al. (2005).

In vitro fermentation test

An experiment using an *in vitro* gas production system (Fedorak and Hurdy, 1983) was conducted to determine rumen fermentation of alfalfa hay and barley grain supplemented with different doses of PBP extracts. One day prior to the experiment, 250 mg of the fermentation substrates (alfalfa hay as roughage-based diet and barley grain as concentrate based diet) were weighed and added into serum bottle (50 ml) in triplicate and then amounts of extracts obtained from 0, 125, 250 and 375 mg of PBP were added to the bottles containing 250 mg of each substrate. On experiment day, rumen fluid was collected using an electric vacuum pump two hours after feeding, from four sheep adapted for two weeks to a TMR ration including 40% alfalfa, 20 % straw wheat and 40 % a commercial concentrate.

The rumen fluid was buffered using McDougall buffer (McDougall, 1948), and 50 ml of this mixture was dispensed into prepared serum bottle containing samples. All bottles were stoppered, crimped and incubated in one run for 24 h at 39° C with constant shaking at 50 × *g*. At the end of incubation period, the gas pressure was measured using a pressure transducer, as well as concentrations of VFAs determined by gas chromatography (Chrompac B.V. CP9002, the Netherlands and Forte GC Capillary Column BP21, SGE Forte GC, UK) using crotonic acid as an internal standard (Playne, 1985). The Ammonia-N concentration of rumen fluid was determined by the phenol–hypochlorite procedure of Broderick and Kang (1980). Ruminal methane (CH_4) calculated by using VFAs proportions according to Moss et al. (2000) as follow: CH4 production = 0.45 (acetate)-0.275 (propionate) +0.4 (butyrate).

Statistical analysis

All variables were statistically analyzed using the general linear model (GLM) procedure of the Statistical Analysis Systems (SAS, 2003), in a completely randomized design. The treatments responses in gas production, VFAs, methane and ammonia-N concentrations were examined in separate models with treatment as a factor. Each factor had three observations (i.e., separate bottle). The least significant difference (LSD) was used to compare the treatments with the respective controls, and significant differences were declared at $P<0.05$. The following statistical model was used:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where Y_{ij} was the dependent variable, μ was the overall mean for each parameter, τ_i was the effect of treatment and ε_{ij} was the residual error.

Results

Chemical composition

The mean of the chemical compositions of pistachio by-product, alfalfa hay and barley grain as substrate samples are shown in Table 1. Pistachio by-product contained 13.24 % CP, 25.97 % NDF, 18.73% ADF and 12.25 % ash. Alfalfa hay and barely grain were used as a forage and concentrate source of the medium, respectively, which differed substantially in content of CP (14.37 vs. 9.37% of DM) and NDF (44.60 vs. 22.20 % of DM).

Efficiency of extraction

The efficiency of PBP extraction using different solvents

Table 1. Chemical composition of pistachio by-product and alfalfa hay and barley grain as substrates

Feeds	Chemical composition (% of DM)				
	Dry matter	Crude protein	Neutral detergent fibre	Acid detergent fibre	Ash
Pistachio by- product	94.35 ± 0.44	13.24 ± 0.19	25.97 ±0.44	18.73± 0.37	12.25± 0.27
Alfalfa hay	95.62± 0.19	14.33± 0.11	44.60± 0.47	31.40± 0.31	12.56± 0.19
Barley grain	95.30± 0.41	9.37± 0.20	22.20± 0.36	6.40± 0.29	3.35± 0.24

and amounts of PC and TT content in PBP and its extracts by different solvents are shown in Table 2. Total extraction yield including water, ethanol 70 %, methanol 80%, and a mixture of methanol and chloroform (50:50) were 34.19, 32.17, 29.99 and 12.03 %, respectively (P<0.01). The total extracted phenolic compounds were 36.96, 65.78, 67.02 and 8.85% and total extracted tannin contents were 37.11, 59.64, 56.87 and 7.55% when PBP was extracted with water, ethanol 70%, methanol 80% and a mixture of chloroform and methanol, respectively.

In vitro fermentation of PBP extracts

The *in vitro* fermentation parameters varied among the AH treatments (Table 3). The inclusion of PBP extracts to AH based substrate significantly (P<0.01) increased gas production after 24 h of incubation. Ruminal ammonia concentrations varied between 49.3 to 81.3 mg/l. In some of the treatments, the addition of mid-level of water PBP extract, high level of ethanol extract, mid and high levels of methanol PBP extracts and low level of a mixture of chloroform and methanol caused a significant

Table 2. Efficiency of PBP extraction and concentration of phenolic compounds and total tannin in PBP and its extracts using different solvents

Item	Pistachio by-product	Extracts from different solvents			
		Water	Ethanol 70%	Methanol 80%	Methanol and chloroform
Efficiency of extraction (%)	-	34.19^{a}± 1.72	32.17^{ab}± 0.52	29.99^{b}± 0.51	12.03^{c}± 0.20
Phenolic compounds (%)	16.35± 0.39	17.52^{c}± 1.07	33.47^{b}± 1.02	36.53^{a}± 1.44	12.03^{d}± 0.90
Total tannin (%)	9.70± 0.49	10.51^{b}±0.67	17.98^{a}± 1.31	18.39^{a}±1.06	6.09^{c}± 1.12

a, c Means within a row with common subscript (s) do not differ (P>0.05).

Table 3. Fermentation products of alfalfa hay supplemented with pistachio by-product extracts in an *in vitro* system

Treatments		Fermentation parameters[1]					
Solvents	Concentrate on extracts[2] (mg)	Gas (ml/g DM)	NH_3-N (mg/L)	VFA (mmol/L)	A:P	CH_4 (mol/100mol)	CH_4 (mol/ mol VFA)
Control (AH)		158.4^{f}	68.2^{cde}	89.1^{g}	2.49^{b}	21.33^{g}	0.239^{cd}
Water	42.5	174.4^{cde}	68.7^{cde}	91.9^{ef}	2.72^{a}	23.05^{e}	0.251^{ab}
	85.0	189.6^{b}	57.7^{fg}	100.9^{ab}	2.83^{a}	24.90^{ab}	0.247^{abc}
	128.5	209.7^{a}	63.0^{cde}	96.4^{d}	2.83^{a}	24.32^{bcd}	0.252^{a}
Ethanol 70%	40.0	162.7^{ef}	81.3^{a}	100.2^{bc}	2.73^{a}	23.69^{cde}	0.236^{d}
	80.0	177.0^{cd}	75.0^{abc}	101.7^{ab}	2.77^{a}	25.13^{ab}	0.247^{abc}
	120.0	178.2^{c}	56.6^{fg}	102.9^{a}	2.77^{a}	24.56^{abcd}	0.239^{cd}
Methanol 80%	37.5	165.7^{def}	72.0^{bcd}	90.0^{fg}	2.70^{a}	21.98^{fg}	0.244^{abcd}
	75.0	165.9^{def}	56.0^{fg}	97.8^{d}	2.82^{a}	23.52^{ed}	0.241^{cd}
	112.5	170.3^{cdef}	49.3^{g}	93.9^{e}	2.76^{a}	22.75^{ef}	0.242^{bcd}
Methanol: chloroform (50:50)	15.0	134.0^{g}	61.2^{f}	98.4^{cd}	2.73^{a}	23.75^{cde}	0.241^{cd}
	30.0	135.4^{g}	78.9^{ab}	100.5^{bc}	2.85^{a}	25.49^{a}	0.253^{a}
	45.0	128.5^{g}	79.6^{ab}	100.3^{bc}	2.78^{a}	24.62^{abc}	0.245^{abcd}
SEM		0.65	0.28	0.72	0.063	0.332	0.0029
p-value		0.0001	0.0001	0.0001	0.0002	0.0001	0.002

[1]VFA= total volatile fatty acid; A: P= Ratio of Acetate to Propionate.
[2]All amounts of extracts in the column were obtained from 125, 250 and 375 mg of PBP respectively.
a, g Means within a column with common subscript (s) do not differ (P>0.05).

Table 4. Fermentation products of barley grain supplemented with pistachio by-product extracts in an *in vitro* system

Treatments		Fermentation parameters[1]					
Solvents	Concentrate on extracts[2] (mg)	Gas (ml/g DM)	NH_3-N (mg/L)	VFA (mmol/L)	A:P	CH_4 (mol/100mol)	CH_4 (mol/ mol VFA)
Control (BG)		199.5^{gh}	57.7^{a}	89.1^{g}	2.29^{cde}	20.41^{e}	0.228^{def}
Water	42.5	210.8^{def}	41.4^{bc}	91.9^{ef}	2.55^{a}	24.05^{a}	0.242^{a}
	85.0	224.4^{b}	36.4^{bcde}	100.9^{ab}	2.35^{bcde}	23.10^{bcd}	0.236^{abc}
	128.5	232.3^{a}	30.3^{de}	96.4^{d}	2.45^{ab}	23.08^{ab}	0.239^{ab}
Ethanol 70%	40.0	208.1^{ef}	31.8^{de}	100.2^{bc}	2.28^{cde}	21.79^{bcde}	0.231^{cde}
	80.0	222.8^{bc}	17.7^{f}	101.7^{ab}	2.21^{e}	22.14^{bcd}	0.228^{def}
	120.0	223.7^{bc}	13.1^{f}	102.9^{a}	2.24^{de}	21.96^{bcde}	0.230^{cdef}
Methanol 80%	37.5	211.0^{def}	27.6^{e}	90.0^{fg}	2.41^{bc}	22.53^{bc}	0.233^{bcd}
	75.0	215.7^{cde}	32.6^{cde}	97.8^{d}	2.36^{bcde}	21.17^{cde}	0.231^{cde}
	112.5	217.9^{bcd}	29.9^{de}	93.9^{e}	2.39^{bcd}	21.85^{bcde}	0.232^{cde}
Methanol : chloroform (50:50)	15.0	206.3^{fg}	38.9^{bcd}	98.4^{cd}	2.30^{cde}	20.90^{ed}	0.227^{def}
	30.0	209.1^{ef}	45.6^{b}	100.5^{bc}	2.23^{e}	21.20^{cde}	0.224^{f}
	45.0	196.9^{h}	38.0^{bcd}	100.3^{bc}	2.26^{cde}	21.05^{cde}	0.225^{ef}
SEM		0.517	0.29	0.72	0.044	0.481	0.0021
p-value		0.0001	0.0001	0.0001	0.0004	0.0001	0.0001

[1]VFA= total volatile fatty acid; A: P= Ratio of Acetate to Propionate.

[2]All amounts of extracts in the column were obtained from 125, 250 and 375 mg of PBP respectively.

$^{a, g}$ Means within a column with common subscript (s) do not differ (P>0.05).

cant reduction (P<0.01) in ruminal ammonia compared to control.

The concentrations of VFAs were higher (P<0.01) with all solvent extracts compared to control group except low level of methanol PBP extract. Similarly, the addition of all solvent extracts caused a significant increase (P<0.01) in the molar proportions of acetate (unreported) and acetate to propionate ratios compared to the control. Except for low level of methanol 80%, methane production increased in all doses of PBP extracts supplementation.

The *in vitro* fermentation parameters with barley grain (BG) treatments are shown in Table 4. Addition, all of the solvent, extracts to BG caused a significant increase (P<0.01) in gas production compared to the control. The ruminal ammonia concentrations ranged between 13.1 to 57.7 mg/l, and all values were lower (P<0.01) in PBP group compared to the control. The lowest ruminal ammonia concentration was observed by high level of ethanol PBP extract.

The results showed that addition of low level of methanol PBP extract did not affect VFAs, but the concentrations of VFAs with other extracts compared to control were higher (P<0.01). Acetate to propionate ratio increased (P<0.01) with the addition of low and high levels of water PBP extracts. However, methane production increased with all levels of water PBP extracts, mid-level of ethanol PBP extract and low level of methanol PBP extract.

Discussion

Chemical composition

The chemical composition of PBP in the current study were consistent with the results of previous reports (Mokhtarpour et al., 2012; Ghaffari et al., 2014b; Shakeri, 2016). The reason for using two different types of the substrate here in this experiment was different responses of PC on rumen fermentation upon the types of substrate fed to the ruminants (Patra, 2011).

The amounts of PC and TT content in PBP were 16.35 and 9.70 % respectively. These values were slightly higher than those reported previously (Mokhtarpour et al., 2012; Ghaffari et al., 2014b; Shakeri, 2016), which may be explained by the differences in plant varieties, growing conditions, climate zone, cropharvesting and processing management (Frutos et al., 2004).

Efficiency of extraction

The highest and the lowest extraction yield contents were observed in water, and a mixture of methanol and chloroform PBP extracts respectively (34.19 % vs. 12.03 %). The extracts yields of the plants materials are strongly dependent on the nature of extracting solvent because of solubility or insolubility of chemical charac-

teristics and polarities (Dai and Mumper, 2010). However, phenolic compounds are often extracted in higher amounts in more polar solvents compared to other solvents (Sultana et al., 2007). According to Markom et al., (2007), Snyder's polarity indexes for ethanol 70%, methanol 80%, a mixture of chloroform and methanol and water were 6.3, 7.1, 5.4 and 9.0, respectively. Phenolics include wide range of compounds with different structure and are often polar, however, due to non-polar molecules linkage, they may be better extracted in low polarity solvents (Dai and Mumper, 2010). Thus, PBP extract is probably often polar with some non-polar groups, hence the highest to lowest amount of extracts were observed with water, ethanol 70 %, methanol 80%, and a mixture of methanol and chloroform. Similarly, Mokhtarpour et al. (2014) reported that extraction of PC from PBP was reduced by increasing the polarity index of the solvents.

In vitro fermentation of PBP extracts

In both substrates, the inclusion of PBP extracts resulted a significant increase ($P<0.01$) in gas production, which might be due to increasing nutrient concentrations in the cultures associated with extracts. The main portion of chemical composition in dried PBP is non-fiber carbohydrates (NFC) with the average content of 430 g/kg (Ghaffari et al., 2014b; Sedighi-Vesagh et al., 2015), which may be related to the result of the gas production through the fermentation of NFC.

Tannins have the ability to bind with protein and decrease proteolysis of feed protein, thus reducing ammonia production (Frutos et al., 2004; Min et al., 2005). This hypothesis was supported by low ammonia-N concentration observed with the addition of PBP extracts to each substrate. These findings were consistent with results of Jolazadeh et al. (2015), who reported lower ruminal ammonia in Holstein bulls fed diets containing different levels of PBP extracts in comparison to control diet. Flythe and Kagan (2010) demonstrated that soluble PC such as isoflavones from red clover has antimicrobial activity against hyper-ammonia-producing bacteria which is responsible for deamination of amino acids in the rumen. As PBP is rich in isoflavones (Tomaino et al., 2010), lower ruminal ammonia substrates supplemented with extracts may be due to the effect of these plant secondary compounds along with the effect of tannins. Furthermore, the effects of PBP extracts on ammonia production were greater for BG based diets than AH based diets. This observation is consistent with other researchers' findings which suggested that when PC were added to herbage than concentrate, they have a milder effect on ruminal fermentation (Vasta et al., 2009: Shakeri et al., 2017). A few researchers evaluated the effect of type of feed (herbage vs concentrate) with or without addition of PC or tannin on ruminal fermentation characteristics and they mainly focused on ruminal biohydrogenation and meat quality (Vasta et al., 2009; Priolo et al., 2009). The fact that tannins exerted a stronger effect when supplemented in the concentrate than in the forage can be explained considering the following factors: in the AH, CP content was 14.33%, whereas in the BG, it was 9.37%. Therefore, more substrate was available for proteolytic bacteria and then for hyper-producing-bacteria. Furthermore, the NDF content of the AH was greater than the BG and thus the cellulolytic bacteria proliferate to a greater extent in forage based diet compared to concentrate based diet. This could promote ruminal fermentation and counteracted the negative effect of PBP tannins on microorganisms' activity. Also, it is possible that, in the BG based substrate, the pH of the medium was lower than the AH, although we did not measure the pH. This low pH along with the PBP tannin extract might exert a stronger inhibiting effect on rumen microbial activity, especially cellulolytic bacteria.

The PBP extracts stimulated some favorable pathways such as VFAs production, which caused a significant increase in acetate to propionate ratio. These findings were inconsistent with results obtained by Jolazadeh et al. (2015) who reported that addition of different levels of PBP extract to the Holstein bull diets did not affect VFAs production and acetate to propionate ratio. It has been reported that lower ruminal microbial activity in the presence of tannins and the lower rate of carbohydrate digestion especially that of fiber may decrease total VFA concentrations in the rumen (Patra and Saxena 2011). On the other hand, the capacity of ruminal microorganisms to degrade plant secondary metabolites and utilizing them as a source of energy may lead to an improvement in nutrient digestibility and VFAs production (Salem et al., 2011). Moreover, rumen bacteria can degrade phenolic compounds such as gallic acid, pyrogallol, phloroglucinol rutin, naringin, and quercetin and flavonoid ring to acetate and butyrate (Patra and Saxena, 2011). Therefore, higher VFAs concentration and A:P ratio in PBP extracts' treatments can be attributed to the combination of the effects discussed above.

Methane production did not decrease by PBP supplementation with extracts in both substrates. The results were consistent with those of Beauchemin et al. (2007), who observed that supplementing 1 and 2% quebracho tannin extract in cattle diets did not affect methane production. There is limited information about the effects

of PBP and PBP extract on methane production in the rumen. However, some reports have identified the significant potential of natural plant extracts as novel sources for methane mitigation from ruminants (Woodward et al., 2002; Shakeri et al., 2017). The PBP extracts tested in this study appeared to have these attributes for use in ruminant nutrition to suppress ruminal ammonia, but no positive effect was found on methane production. However, it must be noted that long-term effects might be different because of the rumen microbes' adaptation.

Conclusions

The results of the current study showed that some of PBP extracts reduced ruminal ammonia and had beneficial effects on ruminal fermentation. However, long-term feeding trials in animals need to be carried out to determine their effects on animal response.

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تأثیر عصاره‌های استخراج‌شده از محصول فرعی پسته بر تخمیر شکمبه و تولید متان

پ. شاکری[1*]، ع. مداحیان[2] و ا. مختارپور[3]

[1]بخش تحقیقات علوم دامی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان کرمان، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرمان، ایران.

[2]دانشکده کشاورزی، دانشگاه پیام نور، ایران.

[3]عضو هیات علمی پژوهشکده دام‌های خاص دانشگاه زابل، زابل، ایران.

[*]نویسنده مسئول، پست الکترونیک: pirouz_shakeri@yahoo.co.uk

چکیده این مطالعه با هدف بررسی تخمیرپذیری شکمبه‌ای یونجه و دانه جو بر اثر افزودن سطوح مختلف عصاره‌های محصول فرعی پسته در شرایط آزمایشگاهی انجام شد. حلال‌های آب، اتانول ۷۰ درصد، متانول ۸۰ درصد و مخلوطی از کلروفرم و متانول به‌ترتیب ۳۶/۹۶، ۶۵/۷۸، ۶۷،۰۲ و ۸/۸۵ درصد از ترکیبات فنلی و ۳۷/۱۱، ۵۹/۶۴، ۵۶/۸۷ و ۷/۵۵ درصد از تانن موجود در محصول فرعی پسته را از طریق عصاره‌گیری استخراج کردند. افزودن عصاره مخلوط کلروفرم و متانول به یونجه و دانه جو تولید گاز را کاهش ($P<۰/۰۱$) داد. اکثر عصاره‌های مورد استفاده در این آزمایش با هر دو سوبسترا، سبب کاهش معنی‌داری در غلظت آمونیاک شکمبه‌ای در مقایسه با گروه شاهد شدند ($P<۰/۰۱$)، در حالی‌که اثر عصاره‌ها بر کاهش تولید آمونیاک با سوبسترای جو بیشتر از یونجه بود. غلظت بیشتری از اسیدهای چرب فرار با افزودن تمام سطوح عصاره‌ها (به‌جز سطح پایینی عصاره متانولی) به هر دو سوبسترا در مقایسه با گروه‌های شاهد تولید شد ($P<۰/۰۱$). علاوه بر این، با افزودن عصاره‌ها به سوبستراها در مقایسه با گروه‌های شاهد، کاهش تولید متان مشاهده نشد. به‌طور کلی، برخی از عصاره‌های مورد استفاده در این آزمایش تأثیر مطلوبی بر فرا سنجه‌های تخمیری شکمبه نظیر تولید گاز، آمونیاک شکمبه‌ای، غلظت اسیدهای چرب فرار داشتند و می‌توان از آن‌ها به عنوان افزودنی‌های خوراکی جدید برای کاهش دفع نیتروژن در نشخوارکنندگان استفاده کرد.