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Evaluating rumen fermentation kinetics and nutritive value of sunflower seed meal supplemented with acetonic extract of pomegranate peel using *in vitro* gas production technique

Shabnam Nezarati, Naser Maheri-Sis*, Abolfazl Aghajanzadeh-Golshani and Ramin Salamatdoust-Nobar

Department of Animal Science, Shabestar Branch, Islamic Azad University, 5381637181, Shabestar, Iran. *Author for correspondence. E-mail: nama1349@gmail.com

ABSTRACT. This study was conducted to investigate effects of supplementing different levels (0.0, 0.5 and 1.0% of buffered rumen fluid) of acetonic extract of pomegranate peel on rumen fermentation kinetics of sunflower seed meal (SFM), using *in vitro* gas production technique. The samples were incubated in syringes containing rumen liquor obtained from three cannulated Iranian Ghezel rams for 2, 4, 6, 8, 12, 24 and 36h. Results indicated that, addition of acetonic extract of pomegranate peel resulted in increase in gas production volume in all of incubation times ($p < 0.0001$). Amount of gas production, also increased by increasing dose of the extract. Amounts of *a* (the gas production from the immediately soluble fraction), *b* (the gas production from the insoluble fraction) and *a* + *b* (the potential gas production) for pomegranate peel extract supplemented sunflower seed meal were higher ($p < 0.05$) than that of control meal. Adding pomegranate peel extract resulted in increase estimated short chain fatty acids (SCFA) production as well as digestible organic matter (DOM), metabolizable energy (ME) and net energy for lactation (NEL) content of SFM. Production of SCFA as well as DOM, ME and NEL content of SFM increased ($p < 0.05$) by enhancing the level of the extract supplementation. In conclusion, it can be suggest that, supplementing acetonic extract of pomegranate peel may be lead to higher ruminal fermentation and better nutritive value of SFM in ruminants.

Keywords: fermentation kinetics; metabolizable energy; pomegranate peel extract, sunflower seed meal.

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Introduction

Banning utilization of protein sources from animal origin in livestock nutrition due to public health and safety considerations resulted in attention of animal nutritionists to using plant protein sources. Sunflower seed meal (SFM) is one of the most utilized alternative oil seed meals in animal nutrition in Iran (Nezarati, Maheri-Sis, Salamatdoust-Nobar, & Aghajanzadeh-Golshani, 2014). However, owing to its higher fiber content than that of other oil seed meals (Morais et al., 2015; Nezarati et al., 2014; National Research Council [NRC], 2001) as well as its higher rumen degradability of protein (Yildiz & Todorov, 2014); it is suggested to limit its maximum inclusion rate in ruminants nutrition.

During recent years, many researchers have emphasized on plant derivatives as rumen fermentation modifiers for improving nutritive value of feedstuffs and consequently productivity of animals (Benchaar et al., 2008; Fugita et al., 2018; Kim et al., 2015; Ornaghi et al., 2017; Tajodini, Moghbeli, Saeedi, & Effati, 2014).

Pomegranate (*Punica granatum* L.) is an ancient medicinal plant with numerous beneficial effects (Zarei, Kafilzadeh, & Shawrang, 2016). Eliyahu et al. (2015) reported that global production of pomegranate fruit was15 million metric tons per year. Derakhshan et al. (2018) cited that Iran contributes in approximately 47% to the total of world pomegranate production. Plentiful proportion of pomegranate fruits processed to producing juice and sauce. Pomegranate peels constitute higher than 50% of the fruit weight. Estimated annual production of pomegranate processing by-products including peels, exceeds 120 thousand tons in Iran (Mirzaei-Aghsaghali et al., 2011; Nezarati & Maheri-Sis, 2016). We have previously studied nutritional value and functional efficiency of pomegranate by-products in ruminants' nutrition (Mirzaei-Aghsaghali et

al., 2011; Nezarati & Maheri-Sis, 2016; Taher-Maddah, Maheri-Sis, Salamatdoustnobar, & Ahmadzadeh, 2012a; Taher-Maddah, Maheri-Sis, Salamatdoustnobar, & Ahmadzadeh, 2012b). Beside pomegranate by products, pomegranate peel possess higher biological activity due to presence of several chemical compounds such as gallic acid, ellagic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, quinic acid, flavon-3-ols and flavonoids, catechin, epicatechin, epigallocatechin-3-gallate, quercetin, aempferol, Luteolin, rutin, kaempferol-3-O-glycoside, kaempferol-3-O-rhamnoglycoside, naringin, anthocyanins, cyanidin, pelarginidin, delphinidin, ellagitannins, punicallin, punicalagin, corilagin, casuarinin, gallagyldilacton, pedunculagin, tellimagrandin, granatin A, granatin B and alkaloids (Taher-Maddah et al., 2012a). These compounds can exert anti-oxidant, anti-bacterial, anti-fungal and anti-protozoal effects (Abarghuei, Rouzbehan, Salem, & Zamiri, 2013; Derakhshan et al., 2018; Fawole, Makunga, & Opara, 2012). Negi & Jayaprakasha (2003) reported that concentration of ployphenolic compounds in acetonic extract of pomegranate peel was higher than that of methanolic and water extract. Abarghuei, Rouzbehan, and Salem (2014) and Abarghuei et al. (2013) stated that addition of pomegranate peel extract in the rumen liquor, resulted in manipulate *in vitro* rumen fermentation products, decrease ammonia nitrogen concentration and protozoa population and increased microbial protein as well as propionic acid concentration and consequently production performance of ruminants. Baladi, Moghaddaszadeh-Ahrabi, and Afrouziyeh (2014) reported that when soybean meal treated with higher doses of tannins extracted from pomegranate pomace, *in vitro* gas production volume and consequently metabolizable energy (ME), net energy for lactation (NEl), digestible organic matter (DOM) content and short chain fatty acids (SCFA) production were reduced. Nezarati and Maheri-Sis (2016) found that *in vitro* supplementation of the pomegranate peel methanolic extract to the buffered rumen liquor of sheep; can be conduce to higher ruminal fermentation and volatile fatty acids production from oil seed meals.

This study was realized to evaluate the effect of supplementing different levels of acetonic extract of pomegranate peel in buffered rumen liquor, on ruminal gas production parameters (*a, b, c* and *a* + *b*) as well as estimated SCFA or volatile fatty acids (VFA) production and DOM, ME and NEl contents of sunflower seed meal using *in vitro* gas production technique.

Material and methods

Sample collection, composition and preparation

Pomegranate fruit has been bought from local market and peels separate from the fruit. Pomegranate peels dried, milled and prepared for extraction. Extraction procedure carried out based on the method described by Patra, Kamra, and Agarwal (2006). Sunflower meal sample of the experiment was obtained from commercial unit in Tabriz, Iran. Collected samples were milled through a 1 mm sieve for chemical analysis and gas production procedure. Chemical compositions of SFM have been determined in the laboratory of feed analysis, Shabestar Branch, Islamic Azad University, based on standard methods (Association Official Analytical Chemist [AOAC], 2005); which dry matter (DM), crude protein (CP), ether extract (EE), Ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) and non-fibrous carbohydrate (NFC) contents were 94.6, 30.0, 5.7, 5.3, 43.2, 31.2 and 15.8%, respectively (Nezarati et al., 2014).

In vitro gas production procedure and estimated values

Rumen fluid required for *in vitro* incubation obtained from three cannulated Ghezel rams fed twice daily with a diet containing mixture of roughage and concentrate (60:40) before the morning feeding. Two hundred mg of dried SFM were weighed in triplicate into 100 ml calibrated glass syringes following the procedures according Menke and Steingass (1988). The syringes were pre-warmed at 39ºC before the injection of 30 mL rumen fluid-buffer mixture (1:2) into each syringe and incubated in an incubator at 39ºC. Samples were incubated in syringes containing rumen liquor taken from three cannulated Iranian Ghezel rams for 2, 4, 6, 8, 12, 24, 36, 48 and 72 hours. Three syringes containing only rumen fluid-buffer mixture considered as the blank. The net gas productions of samples were determined by correcting gas volumes for blanks. Net gas production data were fitted to the exponential model outlined by Ørskov and McDonald (1979) and model components (*a, b* and *c*) calculated by FITCURVE software version 6:

$$Y = a + b(1 - e^{-ct})$$

where Y is the gas production at time t, a is the gas production from soluble fraction (mL 200mg^{-1} DM), b is the gas production from insoluble but fermentable fraction (mL 200mg^{-1} DM), c is the gas production rate constant for the insoluble fraction (mL h^{-1}), a + b the potential gas production (mL 200mg^{-1} DM), t is the incubation time (h) and e is the base for natural logarithms (2.718).

Short chain fatty acids (VFA) were calculated by equation of Makkar (2005):

$$SCFA(mmol) = 0.0222\ GV - 0.00425$$

where, GV is the 24h net gas production volume (mL 200mg^{-1} DM).

Metabolisable energy (ME), digestible organic matter (DOM) and net energy for lactation (NEl) were calculated using the equations of Menke and Steingass (1988) as:

$$ME(MJ\ kg^{-1}DM) = 0.157\ GV + 0.084\ CP + 0.22\ EE - 0.081\ CA + 1.06$$

$$NEl(MJ\ kg^{-1}DM) = 0.115\ GV + 0.054\ CP + 0.14\ EE - 0.054\ CA - 0.36$$

$$DOM(g\ kg^{-1}DM) = 0.9991\ GV + 0.595\ CP + 0.181\ CA + 9$$

where, GV is 24h net gas production volume (mL 200mg^{-1} DM), and CP, EE, CA are crude protein, ether extract and crude ash (g kg^{-1} DM), respectively.

Statistical analyses

Data from *in vitro* gas production test were subjected to analysis of variance as a completely randomized design with three treatments including 0.0, 0.5 and 1.0% of buffered rumen fluid supplemented by acetonic extract of pomegranate peel (three replicates for each treatment), using general linear model (GLM) procedure Statistical Analysis System (SAS, 2004). Means compared by Duncan multiple range tests.

Results and discussion

Gas production volume

Effect of supplementing acetonic extract of pomegranate peel on *in vitro* gas production volume of SFM at different incubation times have been shown in Table 1. Results indicated that, addition of acetonic extract of pomegranate peel led to significant increase in gas production in all incubation times. Also gas production, enhanced by increasing the level of the extract. Amounts of gas produced from control (untreated) SFM at different incubation times approximately comparable with our previous *in vitro* study (Maheri-Sis et al., 2011), but not in agreement with Jolazadeh and Mohammadabadi (2017). Results from current study were partly in line with some other studies (Kim et al., 2015; Nezarati & Maheri-Sis, 2016; Salem et al., 2014).

Salem et al. (2014) notified that addition of extracts obtained from some tree species to a high concentrate diet, linearly increased the *in vitro* gas production at 12, 24, 48 and 72h of incubation. Kim et al. (2015) found that supplementing pomegranate extract results in increasing total gas production at 12, 48 and 72h incubation times as compared with the control. Nezarati and Maheri-Sis (2016) described that addition of methanolic extract of pomegranate peel led to significant increase in total *in vitro* gas production in all incubation times for oil seed meals including SFM. Also they are mentioned that gas production volume enhanced by increasing the dose of the extract. In contrast with our findings, Salamatazar et al. (2011) resulted that, supplementation of sunflower meal by the *thyme* methanolic extract, decreased *in vitro* ruminal gas production. Jolazadeh and Mohammadabadi (2017) proposed that *in vitro* gas production from sunflower meal treated with tannin extracted from pistachio hulls linearly decreased at all incubation times. Baladi et al. (2014) reported that higher doses of tannin extract of pomegranate pomace reduced *in vitro* gas production amount of soybean meal. While, Abarghuei et al. (2014) reported that gas production at 24h incubation time, did not significantly affect by the type and levels of pomegranate peel extract.

Gas production parameters

Effect of acetonic extract of pomegranate peel on *in vitro* gas production parameters (*a, b, c, a* + *b*) of SFM have been shown in Table 2.

Table 1. Effect of addition of acetonic extract of pomegranate peel on *in vitro* gas production volume (mL 200mg^{-1} DM) of sunflower meal at different incubation times (h).

Incubation time (h)	Control (0.0%)	Acetonic extract (0.5%)	Acetonic extract (1%)	*P* value	SEM
2	4.41^{c}	16.35^{b}	20.75^{a}	<0.0001	0.259
4	8.17^{c}	19.49^{b}	25.63^{a}	<0.0001	0.618
6	15.25^{c}	19.96$_{b}$	26.72^{a}	<0.0001	0.759
8	17.45^{c}	28.61^{b}	35.35^{a}	<0.0001	0.776
12	20.44^{c}	33.17^{b}	42.61^{a}	<0.0001	0.782
24	27.36^{c}	41.51^{b}	49.06^{a}	<0.0001	0.618
36	28.30^{c}	44.18^{b}	50.70^{a}	<0.0001	0.453
48	29.72^{c}	48.27^{b}	51.89^{a}	<0.0001	0.494
72	30.66^{c}	52.99^{b}	54.72^{a}	<0.0001	0.341

Means in the same row with different letters (a, b and c) are differ ($p < 0.05$). SEM= standard error of the mean.

Table 2. *In vitro* gas production parameters and estimated VFA production of sunflower meal affected by acetonic extract of pomegranate peel

Item	Control (0.0%)	Acetonic extract (0.5%)	Acetonic extract (1%)	P value	SEM
a	-2.01^{b}	11.80^{a}	12.57^{a}	<0.0001	0.594
b	31.76^{b}	40.33^{a}	40.69^{a}	<0.0002	0.712
c	0.11^{a}	0.05^{b}	0.09^{a}	<0.0019	0.006
a+*b*	29.76^{b}	52.14^{a}	53.26^{a}	<0.0001	0.559
|*a*|+b	33.76^{b}	52.14^{a}	53.26^{a}	<0.0001	0.671
SCFA	0.60^{c}	0.91^{b}	1.08^{a}	<0.0001	0.012
DOM	55.14^{c}	69.28^{b}	76.82^{a}	<0.0001	0.618
ME	8.69^{c}	10.91^{b}	12.10^{a}	<0.0001	0.094
NEl	5.48^{c}	7.11^{b}	7.98^{a}	<0.0001	0.071

Means in the same row with different letters (a, b and c) are differ ($p < 0.05$). a: The gas production from the immediately soluble fraction (ml), b: The gas production from the insoluble fraction (ml), a + b: The potential gas production (mL), *c*: The gas production rate constant for the insoluble fraction *b* (h^{-1}), SCFA: Short chain fatty acids (mmol), ME: metabolisable energy (MJ kg^{-1} DM), NEl: net energy for lactation (MJ kg^{-1} DM), DOM digestible organic matter (% kg^{-1} DM). SEM= standard error of the mean.

There are differences for a (the gas production from the immediately soluble fraction), b (the gas production from the insoluble fraction), *a* + *b* (the potential gas production) and *c* (the gas production rate constant for the insoluble fraction) between treated and untreated SFM. Amounts of *a, b* and a + b for SFM increased by supplementing acetonic extract of pomegranate peel; but there are no differences between different doses of extract addition, view point of *a, b* and *a* + *b*. In view of the fact that, gas production parameters are affected by gas volume; thus, it was predictable that, enhance in gas production resulting to increase in *a, b* and *a* + *b*. However, the parameter *c* unpredictably unaffected by higher dose of the extract comparing untreated SFM, whereas lowered by lower dose of the extract.

The findings of this research are in line with our previous study with adding methanolic pomegranate peel extract on several oil seed meals (Nezarati & Maheri-Sis, 2016). Also, according to the current results, Salem et al. (2014) found that addition of extracts obtained from some tree species to a high concentrate diet, linearly increased the *in vitro* potential gas production (*a* + *b*) and decreased gas production rate (*c*).

Niasati, Palizdar, Pourelmi, and Pasha (2014) and Pashachalandari, Palizdar, Mohammadian-Tabrizi, and Niasati (2014) observed that *in vitro* gas production parameters (a and b) of tested oil seed meals increased while parameter c did not affect or reduced when rumen liquor supplemented with *Urtica dioica* and *Viscum album* extracts. However, Salamatazar et al. (2011) declared that supplementing thyme extract decreased gas production parameters (*a, b, c* and *a* + *b*) for SFM. Baladi et al. (2014) have studied the effects of supplementing tannin extracted from pomegranate pomace on gas production parameters of soybean meal and observed that a + b decreased by higher doses of tannin extract supplementation, whereas parameter *c* remained unaffected. But, Abarghuei et al. (2014) reported that the potential gas production parameter (*a* + *b*), did not affected by doses of pomegranate peel extract, but parameter *c* lowered by supplementing pomegranate peel extract. Jolazadeh and Mohammadabadi (2017) indicated that *in vitro* gas production parameter (*a* + *b*) of sunflower meal treated with tannin extracted from pistachio hulls, reduced by increasing extract doses, but the parameter *c* remained unaffected.

ME, NEl, DOM content and SCFA production

Estimated amounts of ME, NEl and DOM contents of SFM as well as SCFA (VFA) production have been indicated in Tables 2. Short chain fatty acids production and ME, NEl and DOM content of SFM increased (p

< 0.0001) by supplementing acetonic extract of pomegranate peel. Production of SCFA together with ME, NEl and DOM content of SFM were enhanced ($p < 0.0001$) by increasing dose of the extract addition. Energy content and SCFA production of SFM practically is in agreement with Maheri-Sis et al. (2011). Enhancement of estimated amounts of *in vitro* SCFA production is in line with our previous study with adding methanolic pomegranate peel extract on several oil seed meals including SFM (Nezarati & Maheri-Sis, 2016). Salem et al. (2012) explained that plant secondary metabolites have suitable impacts on rumen function owing to their stimulating effect on ruminal fermentation, and enhancing protein metabolism and digestibility of plant cell wall constituents, together with decreasing ruminal methane production and nutritional stresses. These effects finally may be conducing to enhance ME, NEl and DOM content and VFA production of feedstuffs in ruminant animals which happened in current study.

In support of our findings, Demirtaş, Öztürk, and Pişkin (2018) cited that flavonoids can stimulate the fermentative activity of rumen bacteria. For instance, extracts of *Lavandula officinalis* and *Solidago virgaure* have improved the ruminal fermentation via increasing production of VFA and, *Achillea millefolium* increased crude protein and cell wall degradation along with biomass yield. They have noted that, these effects might be owing to conversion of phenolic compounds to more bioactive forms by rumen bacteria and such products in turn, can stimulate the synthesis of aromatic amino acids and enhance the enzymatic activity of some groups of bacteria. Castillejos, Calsamiglia, and Ferret (2006) noted that gram positive bacteria are more sensitive to essential oils than gram negative bacteria; thus, pomegranate peel extracts may have monensin like effects on ruminal metabolism. Jami et al. (2012) concluded that adding pomegranate peel extract to the diet of lactating dairy cattle at the levels of 1, 2 and 4%, resulted in enhancing some bacterial species involved in soluble sugar utilization such as *Succinivibrio dextrinosolvens*, *Eubacterium ruminantium,* and *Streptococcus bovis* and reducing some others which are mainly known as cellulose degrading bacteria such as *Fibrobacter succinogenes* and *Ruminococcus albus*. However, they have observed that cellulose digestion did not reduced and NDF digestibility even increased in the cows fed with highest level (4%) of pomegranate peel extract. Abarghuei et al. (2014) and Abarghuei et al. (2013) have highlighted the anti-protozoa potency of pomegranate peel extract and stated that it may reduce methane production in the rumen and consequently improved rumen energy efficiency. Alternatively, as the protozoa are bacteria predator, decreasing protozoa population can be led to higher bacterial production and conduce to higher microbial protein and VFA production. Moreover, due to antioxidant capacity of phenolic compounds of pomegranate peel, it may acts as free radical scavenger in the rumen and enhances rumen health and microbial growth.

In contrast with our findings, Baladi et al. (2014) declared that ME, NEl, DOM content and SCFA production of soybean meal have reduced when treated by higher but not by lower doses of tannins extracted from pomegranate pomace. Abarghuei et al. (2014) and Abarghuei et al. (2013) reported that total VFA and individual VFA proportions were not affected by supplementing pomegranate peel extract in the diet. But, they have proposed that addition of pomegranate peel extract has decreased protozoa population, ammonia-N concentration, and increased propionate to acetate proportion and microbial protein production. Refat et al. (2015) also found that supplementing high grain diets with pomegranate peel extracts in Rusitec system, results in decreasing total and branched-chain VFA and ammonia nitrogen concentration as well. They have mentioned that, the effects of plant extracts contain high polyphenolic compounds on total VFA production and individual proportion of VFA varied and mostly dependent on source, rate and dosage of polyphenolic compounds. Kim et al. (2015) indicated that addition of pomegranate extract in the rumen liquor could not affect *in vitro* total VFA production and dry matter degradability, but total VFA production were increased or decreased by flavonoid-rich plant extracts according to different incubation times (6, 12, 24, 48, and 72 hours). Jolazadeh and Mohammadabadi (2017) indicated that estimated ME, DOM content and SCFA production of SFM treated with tannin extracted from pistachio hulls, reduced by increasing extract doses. Also higher doses lead to lower pH, NH_3-N and protozoa population.

Variable effects of medicinal plants extracts on ruminal *in vitro* gas production characteristics between various studies, could be referred to dissimilar anti-oxidant, anti-bacterial, anti-protozoal and anti-fungal content of medicinal plants together with different ruminant species, rumen conditions, physical and chemical characteristics of the experimental feedstuffs, rumen microbiota diversity, history of feeds offered to the experimental animals, duration of the experimental period, adaptation period and dosage or

concentration of extracts and extracting procedure and solvent, experimental errors and some other unknown items (Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007; Demirtaş et al., 2018; Nezarati & Maheri-Sis, 2016).

Conclusion

Based on the results of this research, it can be concluded that, supplementing acetonic extract of pomegranate peel to the rumen, can be resulted in higher *in vitro* gas and volatile fatty acids production as well as metabolisable energy, net energy for lactation and digestible organic matter content of sunflower seed meal for ruminants. We could not exactly interpret and justified mechanisms of our findings and further investigations needed for interpreting such results. It is suggested that, in future studies, should considered an additional "control" containing rumen fluid-buffer containing extract, without tested feedstuffs.

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References

Abarghuei, M. J., Rouzbehan, Y., & Salem, A.-F. (2014). The influence of pomegranate-peel extracts on *in vitro* gas production kinetics of rumen inoculum of sheep. *Turkish Journal of Veterinary and Animal Sciences, 38*(2), 212-219. doi: 10.3906/vet-1305-69.

Abarghuei, M. J., Rouzbehan, Y., Salem, A. Z. M., & Zamiri, M. J. (2013). Nutrient digestion, ruminal fermentation and performance of dairy cows fed pomegranate peel extract. *Livestock Science, 157*(2-3), 452-461. doi: 10.1016/j.livsci.2013.09.007.

Association Official Analytical Chemist [AOAC]. (2005). *Official Methods of Analysis* (18th ed.). Gaitherburg, MD: AOAC International.

Baladi, M. R., Moghaddaszadeh-Ahrabi, S., & Afrouziyeh, M. (2014). Influence of the addition of different levels of tannin extracted from pomegranate pomace, on some nutritive value of soybean meal. *European Journal of Experimental Biology, 4*, 148-154.

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G. R., Colombatto, D., McAllister, T. A., & Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology, 145*(1), 209-228. doi: 10.1016/j.anifeedsci.2007.04.014.

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L., & Ferret, A. (2007). Invited review: essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science, 90*(6), 2580-2595. doi: 10.3168/jds.2006-644.

Castillejos, L., Calsamiglia, S., & Ferret, A. (2006). Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow *in vitro* systems. *Journal of Dairy Science, 89*(7), 2649-2658. doi: 10.3168/jds.S0022-0302(06)72341-4.

Demirtaş, A., Öztürk, H., & Pişkin, İ. (2018). Overview of plant extracts and plant secondary metabolites as alternatives to antibiotics for modification of ruminal fermentation. *Ankara Üniversitesi Veteriner Fakültesi Dergisi, 65*(2), 213-217.

Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M. S., ... Sadrabad, E. K. (2018). Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food and Chemical Toxicology, 114*, 108-111. doi: 10.1016/j.fct.2018.02.023.

Eliyahu, D., Yosef, E., Weinberg, Z. G., Hen, Y., Nikbachat, M., Solomon, R., ... Miron, J. (2015). Composition, preservation and digestibility by sheep of wet by-products from the food industry. *Animal Feed Science and Technology, 207*, 1-9. doi: 10.1016/j.anifeedsci.2015.05.005.

Fawole, O. A., Makunga, N. P., & Opara, U. L. (2012). Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. *BMC Complementary and Alternative Medicine, 12*(1), 1-11. doi: 10.1186/1472-6882-12-200.

Fugita, C. A., Prado, R. M., Valero, M. V., Bonafé, E. G., Carvalho, C. B., Guerrero, A., ... Prado, I. N. (2018). Effect of the inclusion of natural additives on animal performance and meat quality of crossbred bulls (Angus vs. Nellore) finished in feedlot. *Animal Production Science, 58*(11), 2076-2083. doi: 10.1071/AN16242.

Jami, E., Shabtay, A., Nikbachat, M., Yosef, E., Miron, J., & Mizrahi, I. (2012). Effects of adding a concentrated pomegranate-residue extract to the ration of lactating cows on *in vivo* digestibility and profile of rumen bacterial population. *Journal of Dairy Science, 95*(10), 5996-6005. doi: 10.3168/jds.2012-5537.

Jolazadeh, A., & Mohammadabadi, T. (2017). Effect of treated sunflower meal with tannin extracted from pistachio hulls on *in vitro* gas production and ruminal fermentation. *Veterinary Research Forum, 8*(3), 203-208.

Kim, E. T., Guan, L. L., Lee, S. J., Lee, S. M., Lee, S. S., Lee, D. I., ... Lee, S. S. (2015). Effects of flavonoid-rich plant extracts on *in vitro* ruminal methanogenesis, microbial populations and fermentation characteristics. *Asian-Australasian Journal of Animal Sciences, 28*(4), 530-537. doi: 10.5713/ajas.14.0692.

Maheri-Sis, N., Baradaran-Hasanzadeh, A. R., Salamatdoust, R., Khosravifar, O., Agajanzadeh-Golshani, A., & Dolgari-Sharaf, J. (2011). Effect of microwave irradiation on nutritive value of sunflower meal for ruminants using *in vitro* gas production technique. *The Journal of Animal & Plant Sciences, 21*(2), 126-131.

Makkar, H. P. S. (2005). *In vitro* gas methods for evaluation of feeds containing phytochemicals. *Animal Feed Science and Technology, 123*, 291-302. doi: 10.1016/j.anifeedsci.2005.06.003.

Menke, K. H., & Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research Development, 28*, 7-55.

Mirzaei-Aghsaghali, A., Maheri-Sis, N., Mansouri, H., Razeghi, M. E., Mirza-Aghazadeh, A., Cheraghi, H., & Aghajanzadeh-Golshani, A. (2011). Evaluating potential nutritive value of pomegranate processing by-products for ruminants using *in vitro* gas production technique. *ARPN Journal of Agricultural and Biological Science, 6*(6), 45-51.

Morais, R. K. O., Silva, A. M. d. A., Bezerra, L. R., Carneiro, H., Moreira, M. N., & Medeiros, F. F. (2015). *In vitro* degradation and total gas production of byproducts generated in the biodiesel production chain. *Acta Scientiarum. Animal Sciences, 37*(2), 143-148. doi: 10.4025/actascianimsci.v37i2.26309.

Negi, P. S., & Jayaprakasha, G. K. (2003). Antioxidant and antibacterial activities of *Punica granatum* peel extracts. *Journal of Food Science, 68*(4), 1473-1477.

Nezarati, S., & Maheri-Sis, N. (2016). Effect of methanolic extract of pomegranate peel on *in vitro* rumen fermentation kinetics of oil seed meals. *Iranian Journal of Applied Animal Science, 6*(1), 67-75.

Nezarati, S., Maheri-Sis, N., Salamatdoust-Nobar, R., & Aghajanzadeh-Golshani, A. (2014). *In vitro* fermentation characteristics and nutritive value of Iranian oil seed meals for ruminants. *Greener Journal of Biological Science, 4*(2), 53-58.

Niasati, M., Palizdar, M. H., Pourelmi, M. R., & Pasha, C. H. (2014). Effect of methanolic extract of *Viscum album* on *in vitro* fermentation and digestibility of soybean meal. *Research Opinions in Animal and Veterinary Sciences, 4*(7), 411-415.

National Research Council [NRC]. (2001). *Nutrient Requirements of Dairy Cattle* (7th rev. ed.). Washington, DC: National Academy Press.

Ornaghi, M. G., Passetti, R. A. C., Torrecilhas, J. A., Mottin, C., Vital, A. C. P., Gurerrero, A., ... Prado, I. N. (2017). Essential oils in the diet of young bulls: Effect on animal performance, digestibility, temperament, feeding behaviour and carcass characteristics. *Animal Feed Science and Technology, 234*, 274-283. doi: 10.1016/j.anifeedsci.2017.10.008.

Ørskov, E. R., & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science, 92*(2), 499-503. doi: 10.1017/S0021859600063048.

Pashachalandari, H., Palizdar, M. H., Mohammadian-Tabrizi, H. R., & Niasati, M. (2014). Effect of methanolic extract of nettle (*Urtica dioica*) on *in vitro* fermentation and gas production of canola meal. *Research Opinions in Animal and Veterinary Sciences, 4*(7), 416-419.

Patra, A. K., Kamra, D. N., & Agarwal, N. (2006). Effect of plant extracts on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. *Animal Feed Science and Technology, 128*(3–4), 276-291. doi: 10.1016/j.anifeedsci.2005.11.001.

Refat, B., Anele, U., He, Z. X., Bassiony, S. M., Abdel-Rahman, G. A., & Yang, W. Z. (2015). Effect of sainfoin hay and pomegranate peel extracts on *in vitro* fermentation and protein degradation using the RUSITEC technique. *Canadian Journal of Animal Science, 95*(3), 417-423. doi: 10.1139/CJAS-2014-142.

Salamatazar, M., Doust-Nobar, R. S., Asadi, Y., Sis, N. M., Najafyar, S., Khodaparast, B., ... Maleki, M. (2011). Effect of thyme methanolic extract on degradability kinetics of sunflower meal. *Annals of Biological Research, 2*, 104-109.

Salem, A. Z. M., Kholif, A. E., Elghandour, M. M. Y., Hernandez, S. R., Domínguez-Vara, I. A., & Mellado, M. (2014). Effect of increasing levels of seven tree species extracts added to a high concentrate diet on *in vitro* rumen gas output. *Animal Science Journal, 85*(9), 853-860. doi: 10.1111/asj.12218.

Salem, A. Z. M., Ronquillo, M., Camacho, L. M., Cerrillo, S. M. A., Domínguez, I. A., & Bórquez, J. L. (2012). Beneficial effects of plant extracts in ruminant nutrition: A review. *Indian Journal of Animal Sciences, 82*(10), 1117-1121.

Statistical Analysis System [SAS]. (2004). *SAS/STAT User guide, Version 9.1.2*. Cary, NC: SAS Institute Inc.

Taher-Maddah, M., Maheri-Sis, N., Salamatdoustnobar, R., & Ahmadzadeh, A. (2012a). Comparing nutritive value of ensiled and dried pomegranate peels for ruminants using *in vitro* gas production technique. *Annals of Biological Research, 3*(4), 1942-1946.

Taher-Maddah, M., Maheri-Sis, N., Salamatdoustnobar, R., & Ahmadzadeh, A. (2012b). Estimating fermentation characteristics and nutritive value of ensiled and dried pomegranate seeds for ruminants using *in vitro* gas production technique. *Open Veterinary Journal, 2*(1), 40-45.

Tajodini, M., Moghbeli, P., Saeedi, H. R., & Effati, M. (2014). The effect of medicinal plants as a feed additive in ruminant nutrition. *Iranian Journal of Applied Animal Science, 4*(4), 681-686.

Yildiz, E., & Todorov, N. (2014). The comparison of the main protein sources for dairy cows: a review. *Bulgarian Journal of Agricultural Science, 20*(2), 428-446.

Zarei, M., Kafilzadeh, F., & Shawrang, P. (2016). *In vitro* gas production and dry matter digestibility of irradiated pomegranate (*Punica granatum*) seeds. *Iranian Journal of Applied Animal Science, 6*(1), 25-34.