Review Article

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Evaluation of pomegranate pomace supplemented with different levels of polyethylene glycol using *in vitro* gas production technique

Abstract

The object of this study was to examine the chemical composition, including the tannin content of pomegranate pomace supplemented with different levels of tannin-binding Agent (Polyethylene Glycol) and gas production amount using *in vitro* gas production technique. The treatment contained 0, 75, 150, 300 and 450mg PEG per each serum bottle, respectively. Approximately 300mg of dried and ground (2mm) Pomegranate pomace were weighed and placed into serum bottles. CP, ADF, NDF, EE, ASH, TP and TT contents in pomegranate pomace were 8.7%, 27.6%, 32.3%, 1.7%, 9.2%, 5.4% and 4.6%, respectively. At the 2 h incubation times, the gas production amount of PP, PP+75mg PEG, PP+150mg PEG, PP+300mg PEG and PP+450mg PEG were 29.83, 32.89, 23.26, 25.83 and 19.37ml/g DM. At the first incubation times (2 and 4h), the PP+75mg PEG treatments had the highest *in vitro* gas production amount within treatments ($P<0.05$). At all incubation times PP+75mg PEG treatment had the highest *in vitro* gas production amount within treatments ($P<0.05$). The addition of PEG at levels 75 and 450mg increased the *in vitro* gas production amount.

Keywords: by-product, *in vitro* gas production, polyethylene glycol, pomegranate pomace, tannin

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Abbreviations: BPF, by-product feedstuffs; PVP, poly vinyl pyrrolidone; PEG, polyethylene glycol; PP, pomegranate pomace; PG, polyethylene glycol; DM, dry matter; CP, crude protein; EE, ether extract; ADF, acid detergent fiber; NDF, neutral detergent fiber; TP, total phenol; TT, total tannins

Introduction

By-product feedstuffs (BPF) obtained from the processing of commercial crops and the food processing industry.[1] Increased disposal costs in many parts of the world lead to increase interest in BPF as alternative feeds for ruminants.[2] Increasing agricultural industrial factories for producing pomegranate juice leads to production of pomegranate peel and the annual production of this by-product approximately 120,000 metric tons in Iran.[3] The pomegranate fruit consists of seeds, the juice and the peels.[4]

A major restriction to increasing livestock productivity in some developing countries is the shortage and fluctuating quantity and quality of the year-round supply of conventional feedstuffs. These countries experience serious shortages in animal feeds of the conventional type. In order to meet high demand for livestock products and to fulfill the future hopes of feeding the millions and safeguarding their food security, the better utilization of non-conventional feed resources which do not compete with human food is imperative. There is also a need to identify and introduce new and lesser known food and feed crops. An important class of non-conventional feeds is BPF which are obtained during harvesting or processing of a commodity in which human food or fibre is derived. The amount of BPF generally increases as the human population increases and economies grow.[1,5,6]

The addition of pomegranate yield by-products in ruminant diets can improve the utilization of low-quality roughages mainly through the supply of protein to rumen microbes, but the presence of tannins in these byproducts prevents not only their optimal utilization but also that of the roughages and byproducts. The addition of a tannin-complexing agent, polyvinylpyrrolidone (PVP), and polyethylene glycol (PEG) to tannin-rich diets is another attractive option to enhance the feeding value of such diets. For about 3 decades, it has been known that tannins bind to PVP and PEG. PVP and PEG are also considered to break already formed tannin-protein complexes, as their affinity for tannins is higher than for proteins. This property of these tannin-complexing agents, in particular of PEG, has been exploited by various workers to alleviate the effects of tannins.[7] The addition of PEG results in the formation of PEG-tannin complexes which inactivates tannins.[8] The PEG may be preferred for inactivation of tannins in feed stuffs as its binding to tannins was highest at near neutral pH values.[7] Addition of PEG to tannin-containing feeds increased *in vitro* gas and SCFA production and *in vitro* degradation of nitrogen. Therefore, there appears to be a potential for improving the utilization of tannin-containing feeds by the use of tannin binding agent such as PEG without altering the genetic pool of tannin-containing plants. Inclusion of energy sources with the aim of synchronizing nitrogen degradability and availability of energy increased the efficiency of microbial protein synthesis in the presence of PEG.[9] This approach can be used both by farmers and by the industry. Farmers can give PEG directly to animals through water, by mixing it with a small amount of concentrate, by spraying it on tannin-rich feed stuffs or better still as a part of nutrient blocks. Industry can incorporate PEG in a pelleted diet composed of ingredients including tannin-rich byproduct(s).[8]

There is little information available regarding the nutritive value of pomegranate pomace (PP) produced in Iran. The aim of this study was to determine the chemical composition, including tannin content of pomegranate pomace supplemented with different levels of tannin-binding Agent (Polyethylene Glycol) and gas production characteristics using *in vitro* gas production technique.

Materials and methods

Pomegranate by-product

Pomegranate pomace (PP) was obtained from fruit juice manufacturing factory of Tabriz, Iran.

Chemical composition

Pomegranate pomace dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE,_method ID 920.30) and crude protein (CP, method ID 984.13) were determined by procedures of AOAC.[10] The NDF and ADF concentrations were determined using the methods of Van Soest et al.,[11] without sodium sulphite. NDF was analysed without amylase with ash included. Total phenolics (TP) were measured using the Folin Ciocalteau method.[12] Total tannin (TT) was determined after adding insoluble polyvinylpyrrolidone and reacting with Folin Ciocalteau reagent.[12]

In vitro gas production trial

The dry matter degradability of each by-product was determined by *in vitro* fermentation with ruminal fluid. Ruminal fluid was collected approximately 2h after morning feeding from two cannulated sheep receiving alfalfa hay, barley and soybean meal. Ruminal fluid was immediately squeezed through four layers of cheesecloth and was transported to the laboratory in a sealed thermos. The resulting ruminal fluid was purged with deoxygenated CO_2 before use as the inoculum. Gas production was measured by Fedorak & Hurdey[13] method. The treatment contained 0, 75, 150, 300 and 450mg PEG per each serum bottle, respectively. Approximately 300mg of dried and ground (2mm) Pomegranate pomace were weighed and placed into serum bottles. There were 3 replicates per treatment. Buffered rumen fluid with McDougal buffer (20ml) was pipetted into each serum bottle.[14] The *in vitro* gas production volume was measured after 2, 4, 6, 8, 12, 16, 24, 36, and 48h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 1g of DM.

Statistical analysis

Data obtained from *in vitro* gas production study was subjected to analysis of variance as a completely randomized design by the GLM procedure of SAS Institute Inc[15] and treatment means were compared by the Duncan test.

Results and discussion

The chemical compositions of pomegranate pomace are shown in Table 1. CP, ADF, NDF, EE, ASH, TP and TT contents in pomegranate pomace were 8.7%, 27.6%, 32.3%, 1.7%, 9.2%, 5.4% and 4.6%, respectively. Chemical compositions of pomegranate pomace in the current study were inconsistent with findings of Taher-Maddah et al.[16] Feizi et al.,[17] reported that DM, OM, CP, crude fiber, and EE values of pomegranate seeds were 94.8, 96.8, 11.4, 38.9, and 1.0%, respectively. These differences in chemical composition of by-products may be due to a difference in cultivar, growing conditions, varieties, and different de-hulling process methods.[16]

Table 1 Chemical composition of Pomegranate pomace (% of DM)

Feedstuff	Items							
	DM	CP	EE	NDF	ADF	ASH	TP	TT
Pomegranate pomace	94.6	8.7	1.7	32.3	27.6	9.2	5.4	4.6

DM, dry matter; CP, crude protein; EE, ether extract; ADF, acid detergent fiber; NDF, neutral detergent fibre; TP, total phenol; TT, total tannins

Gas production volumes (ml/g DM) from *in vitro* incubation of PP supplemented with different levels of Polyethylene Glycol at different incubation times are shown in Table 2 and Figure 1. The volume of *in vitro* gas production increased with increasing time of incubation. Although there are other models available to describe the kinetics of gas production, the Ørskov & McDonald[18] was chosen due to the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs had been documented. Sommart et al.,[19] show that *in vitro* gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Gas volumes also have shown a close relationship with feed intake[20] and growth rate in cattle.[21]

Table 2 Total gas production volume (ml/g DM) in incubation times

Treatments	Incubation times (H)								
	2	4	6	8	12	16	24	36	48
PP	29.83ab	35.53b	40.81c	49.20c	69.64d	89.20d	122.04b	126.47b	132.00c
PP+75mg PEG	32.89a	44.21a	55.33a	81.00a	118.36a	140.14a	148.66a	162.71a	179.30a
PP+150mg PEG	23.26cd	36.37b	47.60b	59.52b	87.69c	114.53b	115.25c	118.87c	120.53d
PP+300mg PEG	25.83bc	34.72b	43.20bc	53.32c	88.32c	101.84c	106.67d	107.56d	108.89e
PP+450mg PEG	19.37d	33.62b	47.73b	76.67a	111.08b	118.91b	122.71b	129.41b	144.45b
SEM	1.57	1.56	1.77	1.41	2.05	1.97	1.64	1.86	1.4

The means within a column without common letter differ ($p<0.05$).

At the 2h incubation times, the *in vitro* gas production amount of PP, PP+75mg PEG, PP+150mg PEG, PP+300mg PEG and PP+450mg PEG were 29.83, 32.89, 23.26, 25.83 and 19.37ml/g DM. At the first incubation times (2 and 4h), the PP+75mg PEG treatments had the highest *in vitro* gas production amount within treatment ($P<0.05$). At the all incubation times PP+75mg PEG treatment had the highest *in vitro* gas production volume within treatment ($P<0.05$). After 48 incubation, the treatments PP+75mg PEG and PP+300mg PEG respectively had highest and lowest *in vitro* gas production among treatments ($P<0.05$).

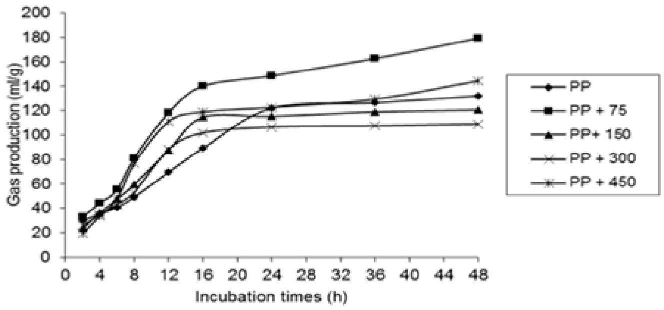

Figure 1 The gas production volume of PP with polyethylene glycol.

Kamalak et al.,[22] reported that total and soluble condensed tannins, NDF and ADF were negatively correlated with estimated parameters of gas production. The results in our study are consistent with those of Feizi et al.,[17] who obtained that tannins of pomegranate peel have negative effect on *in vitro* rumen fermentation. Tannins are considered to have both adverse and beneficial effects in ruminant animals. High concentrations of tannins may reduce intake, digestibility of protein and carbohydrates, and animal performance through their negative effect on palatability and digestion.[4] In the last few years there is an increasing interest of nutritionists in bioactive plant factors-phytofactors as natural feed additives, tannins and etc. that can modify the rumen fermentation processes (e.g., defaunation), improve the protein metabolism and, at the same time, reduce ammonia production and emission, and curb methane production and emission to the atmosphere. High diversity of bioactive phytofactors contained in many plant species has been identified as a potential factor affecting the above-mentioned processes.[23]

The PEG supplementation had significant effect on *in vitro* gas production of PP (Table 2). These results are in agreement with the findings of Getachew et al.,[24] Getachew et al.,[25] Seresinhe and Iben[26] and Singh et al.[27] Tannins lead to form a less digestible complex with crude proteins and may bind and inhibit the endogenous protein, such as digestive enzymes.[28] Tannin can adversely affect the microbial and enzyme activities.[29–32] Hagerman et al.,[33] showed that tannins reduced crude protein digestibility. In another study, McNeill et al.,[14] reported that by increasing condensed tannin in diet, nitrogen digestibility decreased from 0.805 to 0.378 and excretory nitrogen in sheep feces increased from 4.3 to 9.7 g/d. Besharati & s Taghizadeh[5] reported that addition of dried grape by-product to basal diets had effect on digestibility of crude protein ($P<0.05$), also increasing of dried grape by-product supplementation level had linear effect on crude protein digestibility of diets ($P<0.05$). The substantial reduction in nitrogen digestibility as a result of the presence of tannins was similar to that reported in sheep fed *Lotus pedunculatus* as a sole diet[34] and when *Lotus pedunculatus* was fed with ryegrass (*Lolium perenne*),[35] with and without polyethylene glycol. Polyethylene glycol has a high affinity to tannins and makes tannins inert by forming tannin polyethylene glycol complexes.[8] Polyethylene glycol also can also liberate protein from the preformed tannin-protein complexes.[36] The increase in the gas production in the presence of polyethylene glycol is possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen. McSweeney et al.,[37] showed that the addition of polyethylene glycol caused a significant and marked increase in the rate and extent of ammonia production in the rumen.[38,39]

Conclusion

Addition of polyethylene glycol (at levels 75 and 450mg) could overcome adverse effects of tannins on nutrient availability as indicated by gas production parameters. Addition of polyethylene glycol aids inactivated effects of tannins and increased gas production. However there is little information about possibility of using polyethylene glycol in tannin-rich feedstuffs for ruminants. Polyethylene glycol supplementation to improve the nutritive value of pomegranate pomace should be further analyzed in detail: whether or not it is economic due to high cost of polyethylene glycol, before large scale implementation.

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Conflict of interest

The author declares no conflict of interest.

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