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EVALUATING POTENTIAL NUTRITIVE VALUE OF POMEGRANATE PROCESSING BY-PRODUCTS FOR RUMINANTS USING *IN VITRO* GAS PRODUCTION TECHNIQUE

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ABSTRACT

This study was carried out to determine the chemical composition and estimation of nutritive value of pomegranate seed (PS) and pomegranate peel (PP) using *in vitro* gas production technique. Fermentation of PS and PP samples were carried out with rumen fluid obtained from three mature canulated steers. The amount of gas production for PS and PP at 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours were measured. The results showed that gas volume at 24 h incubation (for 200 mg dry samples), were 22.90 and 47.42 ml/200mg DM for PS and PP, respectively. The *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), short chain fatty acid (SCFA) and net energy for lactation (NE_l) contents of PS were 423.4 g/kg DM, 6.20 MJ/kg DM, 0.504 mmol and 2.352 MJ/kg DM, while for PP were 590 g/kg DM, 8.85 MJ/kg DM, 1.048 mmol and 5.092 MJ/kg DM.

Keywords: pomegranate, nutritive value, gas production, short chain fatty acid, metabolizable energy.

Abbreviations

PS	pomegranate seed	PB	pomegranate pulp by-product
PP	pomegranate peel	ADG	average daily gain
CP	crude protein	DM	dry matter
NDF	neutral detergent fiber	EE	ether extract
ADF	acid detergent fiber	BW	body weight
NFC	non-fibrous carbohydrate	DMI	dry matter intake
ME	metabolizable energy	IVDMD	*in vitro* dry matter digestibility
SCFA	short chain fatty acid	VFA	volatile fatty acids
NE_l	net energy for lactation		

1. INTRODUCTION

Climatic conditions and shortage of water resources had increased costs of animal feeds in many countries. Use of agricultural by-products obtained after processing of fruits, vegetables, crops and nuts, such as pomegranate pulp by-product (PB), is often a useful way of overcoming this problem. By-product feedstuffs are becoming increasingly more important in the food and fiber system because they are available for use as livestock feeds at competitive prices relative to other commodities. By-product feedstuffs, which contain little economical value as edible foods for human consumption have become major sources of dietary nutrients and energy in support of milk production and will continue to do so in the future. Many by-products have a substantial potential value as animal feedstuffs. This means that cereals can be largely replaced by these by-products. Consequently the competition between human and animal nutrition and environmental pollution problems can be decreased (Bampidis and Robinson, 2006; Mirzaei-Aghsaghali and Maheri-Sis, 2008a).

Pomegranate (*Punica granatum* L.; Punicaceae) pulp is produced in huge amounts in many parts of the world (Khan, 2009) and in Iran; production of this by-product exceeds 120,000 t/year. The pomegranate pulp by-product consists mainly of peels, stems, and seeds and accounts for about 400-450 g/kg of the weight of the Pomegranate processed into sauce, concentrate and juice (Feizi *et al.*, 2007). Pomegranate peel (PP) attracts attention due to its apparent wound-healing properties, immunomodulatory activity, antibacterial activity, and antiatherosclerotic and antioxidative capacities. Antioxidative activity has often been associated with a decreased risk of various diseases and mortality. A positive correlation between oxidative stress and illnesses is widely documented in cattle (Shabtay *et al.*, 2008). In rats induced to have diarrhea by oral administration of castor oil, feeding extract from pomegranate seeds (PS) decreased defecation and gastrointestinal motility, presumably by reducing fluid pooling into the intestine. To reduce the risk of diarrhea and other diseases, it is critical that calves receive adequate intake of colostrum in the first few hours of life, In addition, the antimicrobial properties of pomegranate extract might directly reduce gastrointestinal infections, which in turn could reduce the risk of diarrhea. Finally, the antioxidant and immunomodulatory properties of pomegranate extract might improve immune function, which could benefit health. Although polyphenolic compounds might improve animal health, they can also decrease proteolytic activity and, thus, compromise protein digestion (Oliveira *et al*., 2010). Feizi *et al*., (2005b) demonstrate the potential of pomegranate seed can be used in animal nutrition. They indicated that inclusion of pomegranate seed up to 25% of the diet has no negative effect on the nutrients intake and digestibility. Also they showed that pomegranate peel tannins have negative effect on *in vitro* rumen fermentation and increase the volume of gas produced with polyvinylpolypyrrolidone, revealed the inhibitory effects of tannins on fermentation (Feizi *et al*., 2005a).

Shabtay *et al*., (2008) demonstrated that PP intake of up to 20% of the total feed intake does not possess deleterious or positive effects on fattening ration intake of feedlot calves. However, because of its palatability, in these amounts, total feed intake is increasing and its nutritional value consequently adds to the average daily gain (ADG).

In vivo, in situ and *in vitro* methods have been used to evaluate the nutritive value of feedstuffs. The *in vitro* gas production technique has proven to be a potentially useful technique to evaluate the nutritive value of feedstuffs, since it gives an estimate of the potential rate and extent of nutrient fermentation in the rumen. However, this technique is measuring gas produced by the fermentation of energy containing components in feeds, and not only that of protein (Mirzaei-Aghsaghali *et al*., 2008b; Maheri-Sis *et al*., 2007). There is, in fact, a lack of information regarding *in vitro* gas production kinetics and nutritive value of PS and PP. Thus, objective of the present research were to determine the nutritive value of PS and PP by-products including chemical composition, *in vitro* gas production characteristics, *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), short chain fatty acids (SCFA) and net energy for lactation (NE_l) by *in vitro* gas production technique.

2. MATERIALS AND METHODS

2.1. Pomegranate seed and pomegranate peel

Randomly fresh PS (after taking juice was obtained) and PP samples was collected from the "San San Shahd" factory in Urmia, Iran. Samples air-dried and ground (1mm and 5mm screen) for chemical analysis and in *vitro* gas production, and evaluated at the laboratories of Animal Science Research Institute in Karaj.

2.2. Chemical Analysis

Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8h and Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein (CP) was calculated as N × 6.25. Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were determined by procedures outlined by Georing and Van Soest (1970) with modifications described by Van Soest *et al*., (1991). Non-Fibrous Carbohydrate (NFC) is calculated using the equation of NRC (2001), NFC = 100 – (NDF + CP + EE + Ash).

2.3. *In vitro* gas production

Fermentation of PS and PP samples were carried out with rumen fluid obtained from three mature canulated steers fed twice daily a diet containing lucerne hay (600 g/kg) plus concentrate mixture (400 g/kg) following the method described by Menke and Steingass (1988). Both solid and liquid rumen fractions were collected before the morning feeding, placed in an insulated plastic container, sealed immediately and transported to the laboratory. Approximately 200 mg PS and PP samples were weighed into the glass syringes of 100 ml. The fluid-buffer mixture (30 ml) was transferred into the glass syringes of 100 ml. The glass syringes containing samples and rumen fluid-buffer mixture were incubated at 39°C. The syringes were gently shaken 30 min after the start of incubation. The gas production was determined at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for PS and PP samples were determined by subtracting the volume of gas produced in the blanks. Gas production data were fitted to the model of Ørskov and McDonald (1979).

$$P = a + b\,(1\text{-}e^{-ct})$$

Where P is the gas production at time t, a the gas production from soluble fraction (ml/200mg DM), b the gas production from insoluble fraction (ml/200mg DM), c the gas production rate constant (ml/h), $a + b$ the potential gas production (ml/200mg DM) and t is the incubation time (h).

The ME and IVOMD contents of PS and PP by-products were calculated using equations of Menke and Steingass (1988) as

ME (MJ /kg DM) = 2.20 + 0.136 × GP + 0.057 × CP

IVOMD (g/kg DM) = 14.88 + 0.889 × GP + 0.45 × CP + 0.0651 × XA

GP = 24 h net gas production (ml/200mg).

CP = Crude protein (g/kg DM)

XA = Ash content (g/kg DM)

Short chain fatty acids (SCFA) is calculated using the equation of Makkar (2005),

Where, Gas is 24 h net gas production (ml/200mg DM).

SCFA (mmol) = 0.0222 × GP – 0.00425

NEL (MJ/kg DM) = 0.115 × GP + 0.0054 × CP + 0.014 × EE - 0.0054 × CA - 0.36 (Abas *et al*., 2005).

Where, GP is 24 h net gas production (ml/200 mg DM), and CP, EE, CA and DOM are crude protein, ether extract, crude ash and digestibility organic matter (g/kg DM), respectively.

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3. RESULTS

3.1. Chemical composition

The chemical composition data of pomegranate by-products are presented in Table-1. The PS had the higher CP content and its concentration was over 150 g/kg DM. The NDF and ADF contents of PS were higher than that of PP, but NFC content of PP was greater than of PS. Generally, the CP, NDF and ADF contents of PS was about 4.27, 3.27 and 3.24 times higher than for PP, while the ash and NFC contents of PP were 2.25 and 5.15 times higher than that of PS, respectively.

3.2. *In vitro* gas production

Gas production volumes (ml/200mg DM) in different incubation times (Figures 1 and 2), gas production parameters (*a*, *b*, *c*) and calculated amounts of IVOMD, ME, SCFA and NE_l of PS and PP are presented in Table-2. Gas volume at 24 h incubation (for 200 mg dry samples), soluble fraction (*a*), insoluble but fermentable fraction (*b*), potential gas production (*a* + *b*) and rate constant of gas production (*c*) of PS were 22.90, 5.69, 49.120, 54.81 ml/200mg DM and 0.0141 ml/h, while for PP were 47.42, 6.729, 47.390, 54.12 ml/200mg DM and 0.0781 ml/h, respectively. Calculated amounts of IVOMD, ME, SCFA and NE_l of PP (590 g/kg DM, 8.85 MJ/kg DM, 1.048 mmol and 5.092 MJ/kg DM, respectively were high as compared to PS (423.4 g/kg DM, 6.20 MJ/kg DM, 0.504 mmol and 2.352 MJ/kg DM, respectively).

4. DISCUSSIONS

4.1. Chemical composition

Crude protein for PS was 154 g/kg DM, a value higher than that of 114 g/kg DM reported by Feizi *et al*., (2007). Ether extract (EE) for PS was 6 g/kg DM, a value lower than that of 1 g/kg DM reported by Feizi *et al*., (2005b). The PP is poor in protein (36 g/kg DM) and rich in tannins (Feizi *et al*., 2007). 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. By preventing bloat and increasing the flow of nonammonia nitrogen and essential amino acids from the rumen, low and moderate (20-45 mg/g DM) concentrations of condensed tannins in the diet improved production efficiency in ruminants, without increasing feed intake (Shabtay *et al*., 2008). 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 (Szumacher-Strabe and Cieślak, 2010). Also, Feizi *et al*., (2007) reported that CP, NDF, ADF, EE, Ash and NFE values of fresh peels were 58, 193, 159, 23, 63 and 713 g/kg DM, while, for ensiled peels were 92, 298, 265, 21, 75 and 581 g/kg DM, respectively. Non-fiber carbohydrate (NFC) level was positively correlated with GP potential. The low non-fiber carbohydrate contents (135 vs. 695.5 g/kg DM) lead to proportionally lower propionate production (Getachew *et al*., 2004). There was a positive correlation between NFC content of feeds and gas production, but feed CP, NH_3-N and NDF levels were negatively correlated with gas production (Getachew *et al*., 2004; Maheri-Sis *et al*., 2007). Different chemical composition leads to different nutritive value, because chemical composition is one of the most important indices of nutritive value of feeds. Variation in chemical components of feeds such as starch, NFC, OM, CP, NDF and soluble sugars contents can be result in variation of *in vitro* gas production volume (Maheri-Sis *et al*., 2008).

4.2. *In vitro* gas production

The cumulative volume of gas production increased with increasing time of incubation. Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) was chosen because the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs had been documented. Sommart *et al*., (2000) reported that 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 (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993). The soluble fraction (*a*) makes it easily attachable by ruminal microorganisms and leads to much gas production (Blummel and Becker, 1997). The microbial ecosystem responds well to the increased supply of readily available carbohydrate and there is an increase in the total bacterial population, fermentation and VFA production - unfortunately this also results in a drop in pH and rumen motility. One consequence of a decrease in motility is a decrease in rumination and less production of saliva leading to a reduction in the buffering capacity of the rumen. However, a sudden increase in rapidly fermentable carbohydrate in the diet may in some instances cause the proliferation of Gram positive organism (S. bovis) which when energy is readily available will switch to the production of lactate (Crichlow and Chaplin, 1985; Nagaraja and Titgemeyer, 2007). The gas volumes at asymptote (*b*) have the advantage for predict feed intake. Blummel and Ørskov (1993) found that gas volume at asymptote could account for 88 % of variance in intake. High rate of gas production (0.0781 vs. 0.0141 ml/h) possibly influenced by carbohydrate fractions readily availability to the microbial population.

It can be see that gas production of PS did not reach a plateau after 48 h fermentation (Figure-1). The

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reason for that, it possibly the carbohydrate fraction have a large proportion of cell walls (680 vs. 208 g/kg DM) and leading to difficulty attached by microorganisms. Cumulative gas volume at each sampling time was affected by variety of feedstuffs. These finding indicate that fraction of substrate and degradability of PS are difference. The gas produced is directly proportional to the rate at which substrate degraded (Dhanoa *et al*., 2000). Additionally, kinetics of gas production is depended on at relative proportions of soluble, insoluble but degraded, and undegradable particles of the feed (Getachew *et al*., 1998). IVOMD for PS was 423.4 g/kg DM, a value lower than that of 447 g/kg DM reported by Feizi *et al*., (2005b). The different result reported by Feizi *et al*., (2005b) about OMD may be due to differences in variety, environment conditions, concentration of cell wall contents, method type for determining OMD (method type in current study was Gas production technique vs. difference method). *In vitro* dry matter and IVOMD were shown to have high correlation with gas volume (Sommart *et al*., 2000). The IVOMD and ME values of PP was lower than reported by Shabtay *et al*., (2008). The difference between the current study and that of reported by Shabtay *et al*., (2008) due to preparation method of PP (dry vs. fresh and ensiled). Low metabolizable energy can be resulted from its low rate of gas production (6.20 vs. 8.85 MJ/kg DM). Menke and Steingass (1988) suggested that gas volume at 24 h after incubation has been relationship with metabolisable energy in feedstuffs.

Pomegranate peel is of high energetic value as compared to elected feedstuffs commonly fed to cattle (NRC 2001), such as alfalfa (ME 8.20 MJ/kg DM), almond hulls (ME 7.91 MJ/kg DM), apple pomace (ME 7.78 MJ/kg DM), barely grain (ME 12.22 MJ/kg DM), barely silage (ME 8.49 MJ/kg DM), citrus pulp (ME 11.54 MJ/kg DM), corn silage (ME 9.74 MJ/kg DM), cotton seed (ME 12.17 MJ/kg DM), sorghum silage (ME 7.49 MJ/kg DM), and tomato pomace (ME 9.91 MJ/kg DM).

The IVOMD decrease in PS than that PP might due to increase in concentration of cell wall contents (680 vs. 208 g/kg DM). It was also illustrated by the negative correlation between ME, IVOMD and IVDMD and the ADF content (r ranging from -0.79 to -0.99) (Happi-Emaga *et al*., 2011).

Ruminants and monogastric herbivores depend on SCFAs for up to 80% and 30-40% of their maintenance energy requirements, respectively. Short chain fatty acids such as acetic, propionic, butyric, isobutyric, valeric, isovaleric, 2-methylbutyric, hexanoic and heptanoic acid, are produced in several parts of the gastrointestinal tract by microbial fermentation of dietary fibre. Acetate, propionate and butyrate, the predominant SCFAs, are readily absorbed and assimilated as a nutrient source by the ruminant. The SCFA account for between 50-70% of digestible energy intake (Mirzaei-Aghsaghali *et al*., 2011). In ruminants, the rumen compartment of the gastrointestinal tract in particular is important for understanding feed digestion and the profile of absorbed nutrients. Because of extensive microbial activity in the rumen, feedstuffs that would be indigestible by the digestive enzymes secreted by the animal are digested by microbial enzymes. In this way as much as 70-80% of largely indigestible feed is converted into microbial organic material (OM; dry matter minus ash) and into volatile fatty acids (VFA) as end-products of microbial fermentation. However, between half and two-third of total metabolizable energy (ME) may originate from VFA produced from fermentative degradation by microorganisms in the rumen. Type of VFA produced is very much dependent on the type of diet, on the type of substrate fermented, and on the fermentation conditions (Bannink, 2007). In addition to their involvement as the major source of energy, the SCFAs also serve as building blocks for milk synthesis; acetate is a necessary component in the formation of milk fat, while propionate is used for glucose production, which is needed for synthesis of milk sugar (lactose). In ruminants, propionate is also the major substrate of hepatic gluconeogenesis. Thus, effective absorption of SCFAs from the forestomach and large intestine is essential for these species (Tagang *et al*., 2010). Getachew *et al*., (2002) reported the close association between SCFA and gas production *in vitro*, and the use of this relationship between SCFA and gas production to estimate the SCFA from gas values, which is an indicator of energy availability to the animal.

The NE_l value of PP was about 2.16 times higher than for PS. The PP is of good NE_l value as compared to elected feedstuffs commonly fed to cattle (NRC 2001), such as alfalfa (NE_l 6.36 MJ/kg DM), almond hulls (NE_l 4.77 MJ/kg DM), apple pomace (NE_l 4.68 MJ/kg DM), barely grain (NE_l 7.78 MJ/kg DM), citrus pulp (NE_l 7.36 MJ/kg DM) and tomato pomace (NE_l 6.36 MJ/kg DM).

Table-1. Chemical composition of pomegranate by-products (g/kg DM, except DM g/kg fresh base).

	DM	CP	NDF	ADF	EE	Ash	NFC
PS	951 ± 0.90	154 ± 8.17	680 ± 12.20	490 ± 7.60	6 ± 0.03	24 ± 0.03	135 ± 7.20
PP	962 ± 0.23	36 ± 0.50	208 ± 3.40	151 ± 2.15	6.1 ± 0.03	54 ± 13.20	695.9 ± 3.4

PS: pomegranate seed, PP: pomegranate peel, DM: dry matter, CP: crude protein, NDF: neutral detergent fiber ADF: acid detergent fiber, EE: ether extract, NFC: non-fiber carbohydrate
Data as: mean± standard deviation

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Table-2. In *vitro* gas production volume (ml/200mg DM) and estimated parameters of pomegranate by-products at different incubation times.

Time (h)	**2**	**4**	**6**	**8**	**12**	**24**	**48**	**72**	**96**
PS	4.00	8.00	9.83	11.90	15.67	22.90	27.00	34.80	44.46
PP	11.67	21.00	25.83	28.90	34.50	47.42	49.50	53.05	57.88
Estimated parameters									
	a	*b*	(*a* + *b*)	*c*	IVOMD	ME	SCFA	NE_l	
PS	5.690	49.120	54.810	0.0141	423.4	6.20	0.504	2.352	
PP	6.729	47.390	54.120	0.0781	590.0	8.85	1.048	5.092	

PS: pomegranate seed, PP: pomegranate peel, *a*: the gas production from soluble fraction (ml/200mg DM), *b*: the gas production from insoluble fraction (ml/200mg DM), *c*: rate constant of gas production during incubation (ml/h), (*a* + *b*): the potential gas production (ml/200mg DM),
IVOMD: *in vitro* organic matter digestibility (g/kg DM),
ME: metabolisable energy (MJ/kg DM), SCFA: short chain fatty acid (mmol),
NE_l: net energy lactation (MJ/kg DM).

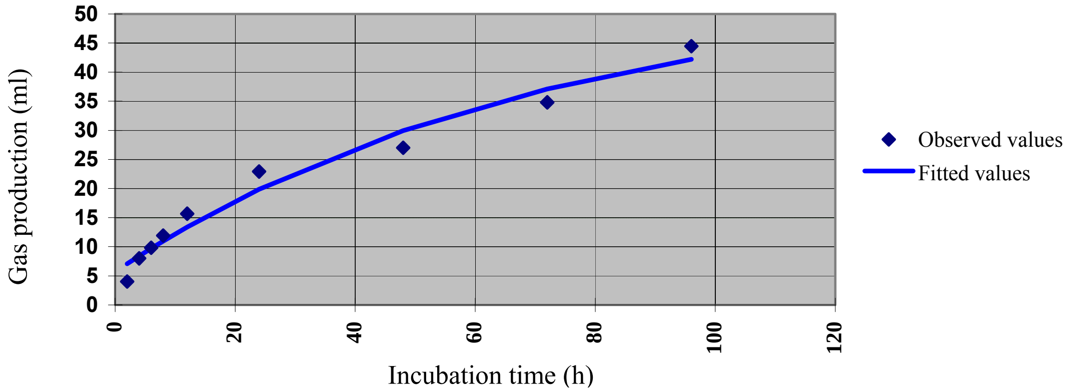

Figure-1. *In vitro* gas production volume of pomegranate seed at different incubation times.

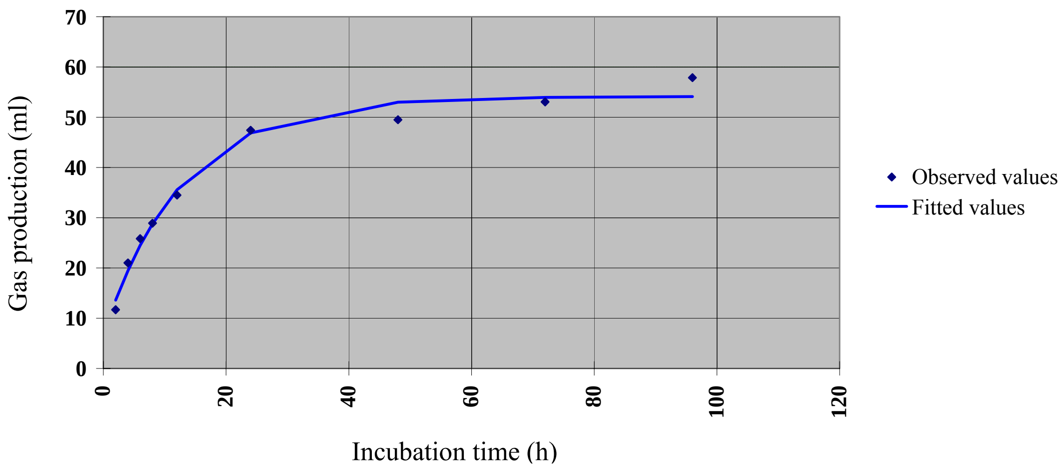

Figure-2. In *vitro* gas production volume of pomegranate peel at different incubation times.

5. CONCLUSIONS

The results of current study based on chemical composition, IVOMD, ME, SCFA and NE_l indicated that pomegranate by-products (PS and PP) could be a fair to good food industrial by-product for ruminant nutrition, but it is required to more research.

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