Determination of Metabolizable Energy of Grape Pomace and Raisin Vitis Leaves Using in Vitro Gas Production Technique

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Abstract. This present study was carried out to determine the fermentation characteristics, metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acids (SCFA), net energy for lactation (NE_l) and volatile fatty acids (VFA) of grape pomace (GP) and raisin vitis leaves (RVL) using gas production technique. Two fistulated whether with average BW 45±2 kg were used. The data was analyzed using completely randomized design. The incubation times were 2, 4, 6, 8, 12, 16, 24, 36, 48 and 72 h. The gas production of soluble and insoluble fractions (a+b) were 289.49 and 249.93 mLg^{-1}DM and the rate of gas production prices (c) were 0.015 and 0.024 (%/h) for RVL and GP, respectively. The ME, OMD, NE_l and VFA for RVL were obtained 15.74 $mjkg^{-1}$DM, 96.97%, 0.0505 Mcal/lb and 2.787 mmol, and for GP were achieved 13.63 $mjkg^{-1}$DM, 87.04%, 0.496 Mcal/lb and 2.484 mmol, respectively, that showed significant differences ($P<0.05$). The amount of gas production in RVL (126.87 mLg^{-1}DM) that was more than GP (112.10 mLg^{-1} DM) resulted low ME in GP compared to RVL. It was concluded, that the GP and RVL can be used in ruminants diets formulation.

Keywords: metabolizable energy, gas production, grape pomace, vitis leaves

Abstrak. Studi ini dilakukan untuk mengetahui karakteristik fermentasi , energi metabolis (ME) , kecernaan bahan organik (OMD) , asam lemak rantai pendek (SCFA) , energi bersih untuk laktasi (nel) dan asam lemak volatil (VFA) anggur pomace (GP) dan daun kismis Vitis (RVL) menggunakan teknik produksi gas . Dua berfistula apakah dengan rata-rata 45 ± 2 BW kg digunakan . Data dianalisis dengan menggunakan rancangan acak lengkap . Waktu inkubasi adalah 2 , 4 , 6 , 8 , 12 , 16 , 24 , 36 , 48 dan 72 jam . Produksi gas dari fraksi larut dan tidak larut (a + b) adalah 289,49 dan 249,93 MLG - 1DM dan tingkat harga produksi gas (c) adalah 0,015 dan 0,024 (% / jam) untuk RVL dan GP , masing-masing. The ME , OMD , NEL dan VFA untuk RVL diperoleh 15,74 mjkg - 1DM , 96,97 % , 0,0505 Mcal / lb dan 2,787 mmol , dan untuk GP dicapai 13,63 mjkg - 1DM , 87.04 % , 0,496 Mcal / lb dan 2,484 mmol , masing-masing , yang menunjukkan perbedaan yang signifikan (P < 0,05). Jumlah produksi gas di RVL (126,87 MLG - 1DM) yang lebih dari GP (112,10 MLG - 1 DM) menghasilkan ME rendah di GP dibandingkan dengan RVL . Disimpulkan bahwa, GP dan RVL dapat digunakan dalam formulasi diet ruminansia.

Kata kunci : inkubasi , produksi gas , anggur pomace , energi metabolis , daun vitis.

Introduction

In middle east, animals suffer from under feeding and malnutrition due to the shortage of locally produced feeds which are not sufficient to cover the nutritional requirements of the animals. Middle east is facing a shortage of compatible sources in ruminant feeds. Therefore, it is necessary that better use of unusual food sources, which are not considered as human foods. Industrial use of agricultural waste, such as citrus pulp, tomatoes and grape pomace can be an important part of the diet of ruminants (Alipour and Rouzbehan, 2007). The annual amount of agricultural by-products produced in Iran is considerable amount. The production of grape by-product in this country is 2.87 million tonnes/year (Besharati and Taghizadeh, 2009). Their use as animal feeds is a means of recycling crop by-products, which, if allowed to accumulate can cause environmental pollution (Huber, 1980). Considerable production of this by-product encourages animal nutritionists to study its nutritive value. Although higher cell wall content of this by-product limits its utilization by non ruminants, it is used widely in ruminant nutrition (Maheri-sis et al., 2012).

Metabolizable energy is part of the general energy that is used by animals for work, growth, fattening up, embryo development, milk production and heat. Metabolizable energy derived by calculating the energy levels in urine and flammable gases (fermentation gases, mainly methane) and the fraction of digestible energy. Urinary energy loss is influenced by two factors: a. Protein quality (biological value), b. Protein intake with energy and protein requirements. If the protein provides essential amino acids are not balanced with the need that it is used to absorb amino acids for protein synthesis, the Disposal will be incomplete and much of the energy body through the urine as urea (Moghaddam, 2010).

Tree leaves are a source of digestible energy, rumen degraded and undegraded protein, vitamins and minerals, there by reducing requirements for concentrates and reducing feeding costs. However, these feeds are generally high in secondary compounds, particularly tannins. Mangan (1988), showed tannins released from one dietary component may react with nutrients in others (Singh et al., 2005). Since the demand for tree fodders in feeding systems generally arise due to the poor quality of available basal forages, tree leaves supplementation strategies should be such that they promote intake and digestibility of basal forages. Since tannins in tree leaves have a wide range of effects on animals, the absence of adequate information on the tannin content of tree leaves, and their influences on rumen digestion, development of appropriate supplementation strategies with them can not be precise. There is little information available on the nutritive value of GP and RVL. Although GP is low in metabolizable energy, it has been used in diets of ruminants fed close to maintenance metabolizable energy levels, especially in sheep (Abel and Icking, 1984). However, inclusion of GP in the diet reduced digestibilities of the diet (Baumgärtel et al., 2007). Yinrong and Yeap Foo (1999), reported that GP tannins have adverse effects on nutrient utilization, and are toxic at high intake levels (Reed, 1995), due to their ability to bind proteins, minerals and carbohydrates (Mc Sweeney et al., 2001). Tannins are the most widely occurring anti-nutritional factor in non-conventional feeds (Besharati and Taghizadeh, 2009).

The aim of this study was to determination of ME, OMD, SCFA, NE_l and VFA of dried GP and RVL using in vitro gas production technique.

Materials and Methods

Dried GP and RVL collection. Grape pomace was obtained from juice production factories of Urmia, Iran. The DGB that was sampled contained grape cluster stems and rejected raisins. Raisin vitis leaves were sampled from farms of Miandoab, Iran.

Chemical composition. Feedstuffs 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 (1999). The neutral detergent insoluble fiber (NDF) and acid detergent fiber (ADF) concentrations were determined using the methods of Van Soest et al. (1991), without sodium sulphite. Neutral detergent insoluble fiber was analyzed without amylase with ash included. Total phenolics were measured using the Folin Ciocalteau method (Makkar, 2000). Total tannin was determined after adding insoluble polyvinyl pyrrolidone and reacting with Folin Ciocalteau reagent (Makkar, 2000). Tannic acid was used as the standard to express the amount of total phenolics and total tannin. Dry matter was determined by drying the treatments at 105°C over night and ashed by igniting the treatments in muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1999). Crude protein was calculated as CP=N×6.25.

Ruminal fluid was collected from two fistulated sheep, about 2 h after their morning feed. Gas production was measured by the method of Fedorak and Hrudy (1983). Approximately 300 mg of ground (2 mm) GP or RVL samples were weighed and placed in to serum bottles (300 mg). The gas production was recorded after 24 h of incubation. Getachew et al. (2002), reported the rate of metabolizable energy each feeds can be achieved using in vitro gas production and chemical composition of each. The metabolizable energy was calculated using of proposed equation by Getachew et al. (2002): ME (mjkg^{-1} DM) = 1.06 + (0.157×GP) + (0.084×CP) + (0.22×CF) - 0.081×CA. The amount of organic matter digestibility was estimated using of proposed equation by Menke et al. (1979), as: OMD (%) = (0.9991×GP) + (0.0595CP) + (0.18×CA) + 9

The short chain fatty acid content calculated using equation: SCFA (mmol) = 0.0222GP – 0.00425. In these equations GP = ml of gas production of 200 mg of dry matter; CP = crude protein on dry matter; CF = crude fat in dry matter; CA = the amount of ash in dry matter.

The VFA were calculated using equation VFA (mmol) = −0.00425 + 0.0222Gv. NE_l was calculated using equation NE_l (Mcal/lb) = (2.20+(0.0272×Gas) + (0.057×CP) + (0.149×CF))/14.64, where gas is 24 h net gas production (mlLg^{-1} DM), Gv = the net gas production (mlL) at 24 h, CP is crude protein (% DM), and CF is crude fat (% DM).

Measuring in vitro gas production. To measure the amount of gas produced from the fermentation of Fedorak and Hurdy (1983), was used. This method is used to calibrate the glass test tubes displacement of water containing rumen fluid and food samples for measurement of gas produced (Fedorak and Hrudy, 1983). 300 mg of each sample mill (with a 2 mm ground) in 50 ml sterile glass cast and was considered four replicates for each sample. About 2 hours after the morning meal to feed the rumen fluid of fistulated sheep, which, fed with a mixture of 60% forage and 40% concentrate diet for a month, collecting and immediately transferred to the laboratory with a smooth layer of fabric in four flasks containing carbon dioxide. Rumen fluid and buffer prepared according to Mc Dougal (1984), the ratio of one part rumen fluid and the second part of the buffer was poured in to the erlen flask and to prevent and reduce the air temperature of fermentation liquid, injecting carbon dioxide in to the mixture at 39° C was placed on the heater temperature. In each sample bottle, 20 ml of the mixture was poured and mixed rumen fluid samples McDougal on feed and after injection of carbon dioxide and anaerobic environment inside the glass, it was tightly closed and placed in the incubator at 19° C with 120 rpm. To correct the gas production with rumen fluid source without the example of four repeated oral (blank) and mixed with 20 ml of rumen fluid and buffer considered and was placed in the incubator. Incubation times were 2, 4, 6, 8, 12, 16, 24, 36, 48 and 72 hours after placing the glass in the incubator and were read to the amount of gas produced Fedorak (liquid handling). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979): $P=a+b(1-e^{-ct})$, where a = the gas production from the immediately soluble fraction (mL), b = the gas production from the insoluble fraction (mL), c = the gas production rate constant for the insoluble fraction (b), t = the incubation time (h) and P = the gas production at the time t.

Statistical analysis. Obtained data from in vitro study was analyzes according to completely randomized design with 4 replicates by the GLM procedure (SAS, 2002). The treatment means were compared by the Duncan test. Statements of statistical significance are based on $P<0.05$.

Results and Discussion

The chemical composition of dried GP and RVL is presented in Table 1. The obtined data for dry matter of test feeds from this study was greater than the values reported (30.5 and 27.3%) by Baumgärtel et al. (2007), those reported (85.45%) by Besharati and Taghizadeh (2009), also was similar data with those values reported by Mirzaei-Aghsaghali et al. (2011), (95.3±0.01% in white GP). The percentage of crude protein of test feeds showed similar values with the data reported (15.5% in red GP) by Baumgärtel et al. (2007), those reported (17.27±0.01% in wihte GP) by Mirzaei-Aghsaghali et al. (2011), also was higher than those values reported by Baumgärtel et al. (2007), (9.3% in white GP).

There were differences between the amounts of acid detergent fiber, neutral detergent fiber, crude protein and ash obtained in this study and the NRC (2001). Total phenolic compounds and total extractable tannins in the leaves of GP and RVL were differed with those reported (6.7 and 5.23%) by Besharati and Taghizadeh (2009), but were consistent with the data reported (2.27 and 1.56% for GP) by Alipour and Rouzbehan (2007). The stage of maturity, types of variety, methods of measurement and dehydration are the factors affecting the phenolic compounds of GP. Regarding to variance in factors influenced chemical composition of GP the crude protein and neutral detergent fibre were in a variety in studied white GP (9.3 and 30.6%), and red GP (15.5 and 50.7%) reported by Baumgärtel et al. (2007).

Gas production influenced is not by any factor other than the chemical and physical properties of food but changes in the microbial activity of rumen fluid may affect the rate of fermentation. The gas production of RVL was more than the GP due to the high concentration of soluble carbohydrates and hemicellulose resulting high ME and degradable. Blümmel and Ørskov (1993) used to describe the fermentation process of gas production technique based on the equation $P=a+b(1-e^{-ct})$. The results showed that the total gas production (a+b) is highly correlated with feed intake (88% DM), digestible intake (93%) and growth rate (95%). Blümmel and Ørskov (1993), reported a correlation equations for dry matter degradation and gas production at different times of incubation in the straw samples. The best equations were obtained which were used when data rumen degradation and gas production after 24 h incubation (R^2=0.97). Means of gas production feeds by incubation at different times is presented in Table 2. As is noted 2 h of incubation the produced gas of RVL was higher that obtained for GP ($P<0.05$). This result may be related to high dry matter in the RVL compared to GP.

The gas production of RVL in rest of incubation times was more than the GP ($P<0.05$), that can be resulted due to the concentration of soluble carbohydrates and high hemicellulose, in resulting high metabolisable energy.

Hemicellulose is effective in gas production conversely, tannins and phenolic compounds that are negative effect on the degradation of proteins and carbohydrates, resulting low gas production.

Cone (1998) showed that the high crude protein feeds, carbon dioxide remains in the liquid and don't out. The creation of this food energy value of gas production is estimated to be less than the actual amount, thus, when different samples of food is high protein, should be corrected in the production of gas ranges with high protein.

Tannins are compounds that bind to proteins because they are out of reach of microorganisms. Consequently, it has limited the growth of microorganisms and gas production is reduced.

Table 1. The chemical composition of feeds (% DM)

Feed	DM	CP	NDF	ADF	OM	CF	HC	TP	TT
GP	96.73[a]	16.59[a]	22.2[b]	20.38[b]	86.75[a]	7.13[a]	1.82[b]	2.715[b]	1.98[b]
RVL	96[b]	14.34[b]	32.02[a]	24.33[a]	85.45[a]	6.23[b]	7.69[a]	4.599[a]	3.548[a]
SEM**	0.0965	0.3010	0.3162	0.398	0.6467	0.1458	0.9128	0.23	0.2074

DM=dry matter, CP=crude protein, NDF=neutral detergent fibre, ADF=acid detergent fibre, OM=organic matter, CF=Crude fat, HC=hemicellulose, TP=Total phenolics, TT=Total tannin, GP=grape pomace and RVL=raisin vitis leaves. Within a column, means without a common superscript letter differ (P< 0.05); **Standard error means of the difference between two treatments means.

Table 2. Means of gas production feeds by incubation at different times in the gas tset method ($mlLg^{-1}$ DM)

Feed	Incubation times (h)									
	2	4	6	8	12	16	24	36	48	72
GP	40.928[b]	50.181[b]	53.908[b]	67.292[b]	75.193[b]	91.90[a]	112.107[b]	142.206[a]	175.926[b]	202.899[a]
RVL	54.248[a]	64.611[a]	85.654[a]	100.037[a]	107.494[a]	114.219[a]	126.87[a]	152.973[a]	197.46[a]	221.991[a]
SEM**	2.472	2.501	3.155	3.559	7.066	6.048	2.06	5.2	3.074	6.308

GP=grape pomace and RVL=raisin vitis leaves; [a,b]Within a column, means without a common superscript letter differ (P< 0.05). **Standard error means of the difference between two treatments means.

Alipour and Rouzbehan (2007) studied the effect of adding polyethylene glycol and the GP silage up on gas and microbial mass production and concluded that the addition of polyethylene glycol increased gas production at all hours. They reported the potential gas production and gas production rate for GP, 223.94 mLg^{-1} DM and 0.04 per h.

The gas production parameters are listed in Table 3. According to our results, RVL had more gas production compared with GP. The results of this study showed that RVL due to more gas production at 24 h had the highest ME, OMD, SCFA, NE_l and VFA.

Besharati et al. (2007), found the gas production and gas production rate of GP about 259.3 mLg^{-1} DM and 0.12 per h. The ME reported values (6.69 $mjkg^{-1}$ DM) of GP by Alipour and Rouzbehan (2007), for those reported (9.78 $mjkg^{-1}$ DM) by Besharati et al. (2007), show is lower than the value obtained in this study according to Pirmohammadi et al. (2007), reports degradation coefficients values of the GP as a, b, c and the effective degradation were 24.3%, 26.3%, 0.04 per h and 35.9%, respectively. Baumgärtel et al. (2007), measured energy values of GP on the weather, which received significant amounts of ME of the white GP (8.3 $mjkg^{-1}$DM) and red GP (5.8 $mjkg^{-1}$DM). The white and red GP in Baumgärtel et al. (2007), experiments emphasized the unpredictable nature of feed with tannins in the nutritional value and other factors. Besharati et al. (2007), reported these values for grape by-product of the 68.9%, 21.8%, 0.0119 per h and 77.1% lower than the obtained results in this study also Pirmohammadi et al. (2007), found the percentage of OMD about 28.5%. This difference in observations could be due to different GP used in experiments and indicate the presence diversity in the by-proudcts. The cell wall of GP used in this study is significantly lower than the percentage of the cell wall of GP (50.4%) used in the study of Pirmohammadi et al. (2007), and is slightly higher (18.7%) used in the study of Besharati et al. (2007). Mirzaei-Aghsaghali et al. (2011), reported amounts of ME (7.40±0.25 MJkg-1 DM), OMD (50.50±1.43 % DM) and SCFA (0.69±0.05mmol) that lower than the obtained results in this study.

Table 3. The parameters estimated from the gas production of feeds

Feed	a	b	c	ME	OMD	SCFA	NE_l	VFA
GP	29.93[b]	220[b]	0.024[a]	13.63[a]	87.04[a]	1.65[a]	0.496[b]	2.484[b]
RVL	54.49[a]	235[a]	0.015[b]	15.74[b]	96.97[b]	1.87[a]	0.505[a]	2.787[a]
SEM**	0.99	2.88	0.00329	0.2887	0.9066	0.0805	0.0012	0.0138

GP=grape pomace, RVL=raisin vitis leaves, ME=metabolizable energy (MJkg-1 DM), OMD=organic matter digestibility (% DM), NEl=net energy for lactation (Mcal/lb) and VFA=volatile fatty acids (mmol), a=gas production potential of the solution (ml), b=gas production potential of the insoluble (ml), c=constant rate of gas production (per h).; [a,b]Within a column, means without a common superscript letter differ (P< 0.05).
**Standard error means of the difference between two treatments means.

In fact, the gas production rate is dependent on the chemical composition of the nutrient, so can be concluded that affected factors including plant species, time of harvest, plant maturity, processing methods and other factors that the nutrient composition and affect the rate of gas production. Gas production is not influenced by any factor other than the chemical and physical properties of food but changes in the microbial activity of rumen fluid may affect the fermentation rate (Menke and Steingass, 1988). Among the factors affecting the results of gas production can be will harvest time, the amount of soluble carbohydrates and insoluble in water, neutral detergent fiber, liquid microbial origin, animal species of the rumen fluid, rumen fluid collection time and ration the rumen fluid.

Conclusion

Regarding to obtained results the GP and RVL have high ME and can be used as energy source in ruminant nutrition. Vitis leaves and grape pomace have anti-nutritional substances containing tannin and phenolic compounds, resulting decreased nutritive value. For introducing of GP and RVL as energy source and determination of suitable concentration in diets required further studies.

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