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Research Paper

A comparison of nutritional value of raisin wastes obtained from two Iranian grape varieties by *in vitro* rumen fermentation

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In vitro cumulative gas production and some laboratory methods were used to evaluate raisin wastes obtained from two Iranian grape varieties (Paykami and Askari) for their nutritive value and chemical composition. Results showed that Paykami raisin wastes (PRW) had numerically more ($P>0.05$) dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE) and ash than Askari raisin wastes (ARW). In another experiment, an *in vitro* gas production (GP) technique was applied to evaluate the nutritive value of raisin wastes. Total gas volume (GP24, GP48 and GP96h after incubation), total volume of gas produced from the insoluble and fermentable section (B_{gas}), constant rate for total gas production (C_{gas}), short chain volatile fatty acids (SCFA), microbial protein yield (MPY), organic matter digestibility (OMD), metabolisable energy (ME) and degradability of dry matter (DMD) were not differ significantly between two treatments. Both treatments had high levels of tannin and phenolic compounds (especially for Askari raisin wastes). Despite having moderate nutritive values for both raisin wastes with high concentration of tannins, the feed consumption of them should be with caution in livestock.

Key words: Raisin wastes, grape, *in vitro*, gas production, tannin

INTRODUCTION

Improvement in animal productions depends on sufficient nutrition due to composition and quality of feedstuff via voluntary intake and digestibility. Evaluation of feedstuffs for fermentability by simple methods (gas production) and their ability to supply nutrients is necessary to adjust a balanced diet in animals. It is necessary to identify and introduce new and lesser known feedstuffs for ruminants. Some by-products are classes of conventional feeds which are obtained during processing of human foods. The annually producing of agro-by-products by Iran country is various, even though, more than 2.05 million ton grape were produced in 2013 (with area harvested of 207 thousand hectares), that section of produced grape is used for raisin production (FAOSTAT, 2013). Raisin wastes are a by-product of the grape treatment. In the grape processing, the production of raising waste (contain of grape cluster and stems plus rejected raisins) is notable. Application of raisin wastes in ruminant diets can improve the utilization of low quality roughages mainly via supply of protein to rumen microorganisms, but the presence of tannins often limits the utilization of feedstuffs (Mangan, 1988; Kumar and Vaithiayanathan, 1990). The wastes from grape processing which contain of seed, skin, pulp and stalk have been studied by nutritionist in animal feedstuffs (Baumgartel et al., 2007; Alipour and Rouzbehan, 2007; Spanghero et al., 2009) as a dietary fiber with polyphenols compounds. However, localized and limited availability of it, variation in nutrient content and contaminants have restricted application of grape by-products in the ruminant and food industries (Spanghero et al., 2009).

The stalk, skin plus pulp, and seed obtained from white grape pomace averagely was 33.2, 34.9 and 31.9 % respectively and These values was 20.7, 41.0 and 38.3% respectively for red grape pomace (Basalan et al., 2011).Yari et al., (2015) reported different nutritive values for each section of raisin by-products (some outer layer of flesh, skin plus pedicle of berries as treatment 1; rejected raisins mostly un-ripped berries with their pedicles as treatment of 2; stalks, rachises plus pedicles of grapevines as treatment of 3), although the treatment 1 had less ADF, Ash and NDF while these values were higher for treatment 3, and with intermediate for treatment 2.

The grape pomace can be useful as a feedstuff with rich soluble carbohydrates in the ruminant diets especially in unsuitable condition which restrict the supplying of other feedstuffs (Basalan et al., 2011). Nutritional value of the grape by-products can be affected by process of wine production, grape variety (i.e. red versus white; Ruberto et al., 2008), and the proportion of seeds, pulp, skin and stalk in the pomace (Baumgartel et al., 2007). So these parameters can change chemical composition and digestibility of grape pomace. Previous studies showed, grape by-products including pomace and raisin wastes have low nutrient availability for ruminants probably as a result of their higher phenolic compounds like tannins (Besharati and Taghizadeh, 2009; Abel and Icking, 1984).

Tannins are a classes of condensed and hydrolysable tannins which have both positive and negative effects in ruminant nutrition (Makkar, 2003). Tannin binding with proteins in the rumen can reach out the proteins from access to digestion of microorganisms and yet decrease ruminally protein degradation and fractional absorption of reached amino acids to the small intestine (McNabb et al., 1996).

The *in vitro* methods have been used to evaluate the nutritive value of feedstuffs by many researchers (Kazemi et al., 2012; Chikagwa-Malunga et al., 2009) and these techniques can be a potentially useful method for feed evaluation, as it is capable of measuring rate and extent of nutrient degradation. There is no general information on nutrient composition and availability of waste raisin in ruminants, so the objective of current experiment was to determine the nutritive value of raisin wastes using *in vitro* gas test and chemical composition analysis.

MATERIALS AND METHODS

Raisin waste preparation

When the grape was sweet, the grape clusters were collected and then soaked with a solution contain of 90 g/kg K_2CO_3+1.5 g/kg olive oil and immediately transferred on a wired network for drying (7-12 days) (Yari et al., 2015). Different raisin wastes were produced during machinery cleaning, sorting and the packing of sun dried treated grape cluster. So whole samples of raisin wastes (contain of stalk, peduncle, pedicel and rejected raisin) were collected from three raisin processing factories (Kashmar city, Razavi khorasan province, Iran) for *in vitro* gas test and chemical composition analysis (Figure 1). The samples of raisin wastes were collected from two different variety of treated grape for raisin production (Paykami and Askari). These varieties planted abundantly in Kashmar city.

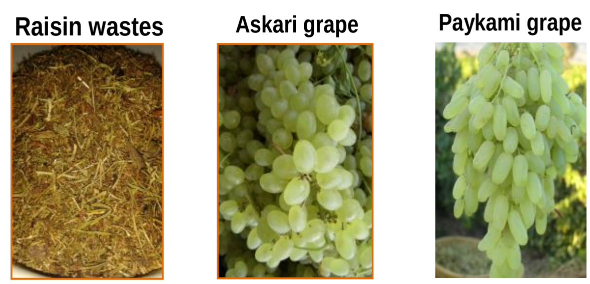

Figure1. The pictures of raisin wastes, Paykami and Askari grapes.

Chemical composition

The chemical composition of Dry matter (DM), crude protein (CP), ether extracts (EE) and Ash of samples was determined by AOAC (1990) methods. The component of Acid detergent fiber (ADF) and Neutral detergent fiber (NDF) were determined by Van Soestet al., (1991) procedure. Non-fiber carbohydrates (NFC) were calculated as Basalan et al., (2011) by following formula, NFC=OM-(CP+EE+NDF). The NTP (phenolic compounds without total tannin) and TP (total phenolic compounds) was measured by Makkar, (2000) and TT (total tannin) were calculated by subtracting NTP from TP.

In vitro gas production procedure

Before the morning feeding, the rumen liquid was collected from three Holstein steers via fistulae (body weight of 450 ± 14 kg). Steers were fed twice daily at 07:30 and 16:30 h with 75% of alfalfa silage and 25% of a pre-prepared concentrate at maintenance and with free access to water. Rumen fluid was filtered through multi layer polyester cloth to eliminate large feed particles and transferred immediately to the laboratory. In an anaerobic condition, 30 ml of rumen fluid plus buffer solution (Menke and Steingass, 1988) was pumped with a bottle top dispenser (Jencons, Hemel Hemstead, England) into a bottle of 120 ml containing 200 mg of the experimental diet. The pressure of produced gas in the bottles was recorded by the barometer ((PTB330, Env Company) according to Theodorou et al., (1994) and Mauricio et al., (1999) (for 2, 4, 6, 8, 12, 16, 24, 48, 72 and 96 h after inoculation). Four blanks were also considered for correction *in vitro* gas production and also 6 samples of alfalfa as slandered were incubated in each run. Once filled up, all the bottles were closed with rubber stoppers, crimped with aluminum seals, shaken and placed in the incubator shaker at 39°C. The samples were incubated with 6 replications and 2 runs.

Calculations and statistical analysis: The organic matter digestibility (OMD) and metabolisable energy (ME) were estimated by the equations of Menke et al., (1979) as: ME (MJ/kg DM)=2.20+0.136×Gp+0.057×CP; OMD (g kg of DM)=(14.88+0.889×Gp+0.45×CP+0.0651×XA)×10 , Where, CP is crude protein of sample(% of DM), XA= ash (% of DM) and Gp is the net gas production (ml) from 200 mg (DM basis) after 24 h of incubation. Short chain fatty acids (SCFA) was calculated by Makkar, (2005) equation as: SCFA (mmol)=0.0222×GP–0.00425 Where, GP is 24 h net gas production (ml/200 mg DM). Microbial protein yield (MPY) was estimated by the equation of Czerkawski (1986) as 19.3 g microbial nitrogen per kg of OMD. Cumulative gas production data were fitted to the exponential equation $y = b (1 - e^{-ct})$ according to ørskov and Mcdonald (1979), where b is the total volume of gas produced from the insoluble and fermentable section (ml); c is constant rate of total gas production from fermentation of b section (ml/h); t is the time of incubation (h) and y is the produced gas at the time of t (ml). A completely randomized design applied for data analysis using the Statistical Analysis System (SAS, 2002) program General Linear Model procedure (SAS, 9.1). The Duncan's test (at P<0.05 levels) was applied for mean comparison of treatment.

RESULTS

Chemical composition

All of the chemical compositions did not differ significantly between two grape variety (Table 1).The DM, CP, NDF, ADF, EE, and ash contents were numerically higher for PRW than ARW, instead TP, NTP, TT and NFC was numerically higher for ARW. The relative contribution for the peduncle, pedicle, and rejected raisin (berries) were an average of 24.65, 57.26 and 18.09 % respectively for PRW and 10.03, 72.41 and 17.56 % respectively for ARW. Whether these differences in composition of raisin may be due to variety variations, their maturity at harvest, the applied processing or separation methods, or belong to other unknown factors.

In vitro gas production procedure and estimated parameters

The parameters of *in vitro* gas production obtained from raisin wastes were showed in (Table 2). The total gas volume for 48, 96 h and B_{gas} was numerically higher for PRW, but the cumulative gas production after 24 h

Table 1. The chemical composition of raisin wastes obtained from two varieties of Paykami and Askari grapes.

	Samples		
Composition (%)	**PRW**	**ARW**	**SEM**
DM	89.63	89.33	0.12
CP	12.28	10.29	1.06
NDF	32.51	31.86	3.44
ADF	26.95	25.91	2.70
EE	1.73	1.68	0.12
Ash	7.46	7.29	0.75
NFC	46.02	48.88	4.60
TP	10.26	14.42	2.02
NTP	8.21	11.34	1.65
TT	2.05	3.08	0.40

[a,b] Means in rows with various superscripts differ at P<0.05.PRW=Paykami raisin wastes; ARW=Askari raisin wastes DM=Dry matter; CP=Crude protein; NDF=Neutral detergent fiber; ADF=Acid detergent fiber; EE=ether extract; NFC=Non fiber carbohydrates; T=Total phenolic compounds; NTP=phenolic compounds without total tannin; TT=Total Tannin.

Table 2. The parameters of *in vitro* gas production obtained from raisin wastes.

	Samples		
Item	**PRW**	**ARW**	**SEM**
B_{gas}	14.85	13.65	0.60
C_{gas}	0.052	0.066	0. 005
$IVGP_{24}$	9.39	9.49	0.50
$IVGP_{48}$	12.64	12.00	0.47
$IVGP_{96}$	15.52	14.77	0.70

[a,b] Means in rows with various superscripts differ at P<0.05. PRW=Paykami raisin wastes; ARW=Askari raisin wastes;
B_{gas} =Total volume of produced gas from the insoluble and fermentable section (ml/200 mg of DM); C_{gas}=constant rate of total gas production from fermentation of b section (ml/h/200 mg of DM); $IVGP_{24}$=total gas produced after 24 h incubation; $IVGP_{48}$=Total gas produced after 48 h incubation; $IVGP_{96}$ = total gas produced after 96 h incubation.

Table 3. Estimated parameters from *in vitro* gas production for raisin wastes.

Item	Samples		SEM
	PRW	ARW	
OMD(g/kg DM)	292.38	284.8	4.43
ME(MJ/kg DM)	4.18	4.08	0.06
SCFA (mmol)	0.20	0.21	0.01
Microbial protein yield(g/kg OMD)	5.64	5.49	0.08

[a,b] Means in rows with various superscripts differ at P<0.05. PRW=Paykami raisin wastes; ARW=Askari raisin wastes, OMD=Organic matter digestibility; ME=Metabolisable energy; SCFA=Short chain volatile fatty acid.

incubation and constant rate of produced gas (C_{gas}) was higher for ARW. The PRW had numerically higher OMD, ME and microbial protein yield, but SCFA was numerically higher for ARW (P>0.05), although this difference is negligible (Table 3).

DISCUSSION

Chemical composition

The chemical composition of two raisin wastes (PRW and ARW) was not significantly affected by grape varieties (Paykami and Askari).There were limited studies about whole raisin wastes, but there is more information about each of component of raisin wastes (for example stalk, pedicles, rachis, peduncles and rejected raisin) (Yari et al., 2015). The average of DM, ADF, NDF, Ash, CP, EE and NFC for all three treatments [some outer layer of flesh, skin plus pedicle of berries as treatment 1; rejected raisins mostly un-ripped berries with their pedicles as treatment of 2; stalks, rachises plus pedicles of grapevines as treatment of 3] reported 96.2, 21.8, 30.3, 5.4, 6.1, 3.0 and 55.1% respectively, while these values were 89.5, 26.4, 32.2, 7.4, 11.3, 1.7 and 47.4% respectively for both Paykami and Askari rasin wastes in this experiment. The CP, NDF, ADF, Ash and NFC of grape pomace reported 17.27, 59.5 and 52.5% respectively (Mirzaei-Aghsaghali et al., 2011). There wasa little data about DM content of raisin wastes. The reported DM (96.2) in Yari et al. (2015) studies was higher than our data that these differences may be as a result of various sampling time and season of harvest. Also maturity of grape, the combination of various components of grapes in raisin wastes after treatment, grape varieties and separation methods may change the DM of raisin wastes and in some cases, also it can alter their chemical composition. The raisin wastes in our study had a more ash content (mean of 7.4 for both raisin wastes) than reports of Can et al., (2004) and Ozduven et al., (2005) with 5.3 and 5.7% respectively, but lower than values of 18.6 from Saricicek and Kilic, (2002) and 10.7% from Alipour and Rouzbehan (2007) for grape pomace. The NDF and ADF contents of grape pomace were 63.0 and 57.0% respectively (Motta Ferreira et al., 1996), notably higher than the mean values of both raisin wastes (32.2 and 26.4%) in our study. Total tannin (2.6%) and total phenolic composition (12.3%) were relatively higher both raisin wastes, but this values was averagely reported by Yari et al., (2015) about 6, 6.6% respectively for all three treatments.

The TT and TP values was numerically higher for ARW than PRW (3.08, 14.42 vs. 2.05, 10.26 respectively). Structural carbohydrates are mostly found in stalk (contain of rachises with their lateral branches and peduncles as supportive tissues), while non-structural carbohydrates (simple sugar, pectin and starch) mostly can be found in the berries (Alipour and Rouzbehan, 2007; Moghaddam et al., 2013). NRC (2001) reported that simple sugar, pectin and starch are classified as NFC. These may be the cause of lower NDF, ADF and higher NFC in ARW and perhaps part of this change is due to variable components of raisin wastes, as ARW samples had higher pedicel (72.41%) and less rejected raising (mainly berries) than PRW. Yari et al. (2015) reported that the raisin wastes contain of stalk (mainly pedicle, rachis) had lower NFC and higher ADF, NDF, Ash and CP contents (p<0.05). Vivin et al. (2003) reported lower CP and more NFC for ripped berries compared with rachises and their lateral branches and peduncles because of increasing the soluble carbohydrates in them that may reduce other compounds like N containing compounds. Yari et al. (2015) reported more carbohydrates for total of external layer of flesh plus skin and pedicle of berries. The ARW had higher total phenol (TP), phenolic compounds non tannin (NTP) and total tannin (TT) concentration compared with PRW (Table 1). Hellman, (2003) reported that the seed and skin in berries of grapevine are the primary source of tannins. The TT and TP were higher for sum of stalks, rachises and pedicles of raisin wastes (6.9, 7.4% vs. 3.5, and 3.7%) than broken raisins and mostly un-ripped berries with their pedicles (Yari et al., 2015). Stalk of grape (contain of rachises with their lateral branches and peduncles, polyphenols) is mostly associated with fiber and lignin are considered as a part of total tannin (Llobera and Canellas, 2007), so Yari et al. (2015) reported that stalks, rachises and pedicles of grapevines with more fiber and lignin might have been resulted in higher TP and TT, but these reports is contrast with our studies. It seems that level of phenolic compounds in raisin wastes depending on the type and the part of raisin tissue. The maximum amount of polyphenols and condensed tannins was observed in the skin of grape wastes (Makris et al., 2007). The processing methods and variety is an important factor influencing the phenolic compounds (Spanghero et al., 2009). So it seems part of differences in phenolic compounds and tannin of raisin wastes is related to variety in this experiment.

In vitro gas production procedure and estimated parameters

Generally, no significant effect observed for cumulative gas production both PRW and ARW($p>0.05$), So it seems grape variety has no effect on cumulative gas production and a little difference between treatments for gas production may be as a result of changes in chemical composition of samples like ADF, NFC, Ash, CP, NDF and tannin. Total gas volume at 24, 48 and 96 h after incubation (200 mg of sample), and constant rate of gas production (C_{gas}) were 30.92, 37.33, 60.30 ml and 0.012 ml/h consecutively (Mirzaei-Aghsaghali et al., 2011), but in our study this parameters was lower [9.39 vs. 9.49 (PRW vs. ARW), 12.64 vs. 12.0, 15.52 vs. 14.77 ml and 0.052 vs. 0.066 ml/h respectively]. Alipour and Rouzbehan (2007) measured *in vitro* gas volume on ensiled grape pomace and showed the higher total gas volume for 24 h after incubation than those reported in the present study (that is 21–23 ml/200mg DM of substrate) for pulps. Some of differences about produced gas volume between our results and other might be as a result of used various sources of grape (for example grape pomace or raisin wastes). Part of metabolisable energy (65-75%) provided by SCFA (Penner et al., 2009). Total gas volume can be related to SCFA (short chain fatty acids) production (The CO_2 and CH_4 in the medium culture is estimable from acetate, propionate and butyrate as volatile fatty acids produced in the medium), so increasing of SCFA led to increase of the digestibility, energy and subsequently increasing of the gas volume from culture medium (Maheri-Sis et al., 2008). Despite the low level of metabolisable energy (ME) for dried or ensiled grape pomace, but it can be used in ruminant's nutrition at maintenance ME levels (Abel and Icking, 1984). The use of grape pomace in the diet decreased the digestibilitiy of it (Baumgartel et al., 2007). Some results indicated that grape pomace had some anti-nutritional factors (such as tannins) that can be toxic at high level of consumption, because they are capable of binding with proteins, minerals and carbohydrates (Reed, 1995; Yinrong and Yeap, 1999; McSweeney et al., 2001). INRA, (2007) and DLG, (1997) reported the ME of 4.2 to 5.4 MJ/kg respectively for grape pomace, so our results (4.08 and 4.18 MJ/kg DM for Askari and Paykami respectively) is near to INRA, (2007) and are lower than those reported by DLG, (1997). However, in our estimation, GP24 for raisin wastes is less to that calculated by Spanghero, (2007) on pulp and seed samples (with mean of 17.56 and 12.06 ml for all treatments).

Conclusion

Nutrient content of raisin wastes differed with grape variety, although these differences were not significant. In general, it seems that raisin wastes obtained from Paykami grapes nutritionally is superior to Askari grapes.

Chemical composition and *in vitro* gas production results indicate that the estimated ME value of raisin wastes is low, even if the variety of grape for raisin wastes has been changed. The levels of tannin and phenolic components in the wastes of raisin used in this experiment was relatively high, so it seems that due to adverse effects of tannins compounds , its use must be controlled. More studies on nutritive value of raisin wastes using *in vivo* technique must be designed in future.

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Authors` declaration

We declare that this study is an original research by our research team and we agree to publish it in the Journal.

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