Prediction of energy contents and potential nutrient supply of raisin byproducts for ruminants using National Research Council feeding system and in vitro gas production method

Article *in* Research Opinions in Animal and Veterinary Sciences · August 2015

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www.roavs.com EISSN: 2223-0343

RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Research article

Prediction of energy contents and potential nutrient supply of raisin by-products for ruminants using National Research Council feeding system and *in vitro* gas production method

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Article history
Received: 30 May, 2015
Revised: 5 Jul, 2015
Accepted: 6 Jul, 2015

Abstract

Using local agro-industrial by-products can help to fill feed shortages in ruminant feeding in semi-arid climate condition. The objective of current study was to determine the nutritive value of several sun dried grapevine (raisin) by-products (RBPs) for ruminants using National Research Council feeding system (NRC) and *in vitro* gas production method. Several RBPs of sun dried treated grapevine cluster (*Vitis vinifera L. cv. Sultana*), were 1) some outer layer of flesh and skin and pedicle of berries (RBP1); 2) rejected raisins mostly un-ripped berries with their pedicles (RBP2) and 3) peduncles and rachises with their lateral branches of clusters (RBP3). Using NRC 1996 and 2001 feeding system, RBP1 and RBP2 had higher predicted metabolizable energy (ME; 11.48 and 9.45 vs. 5.99 MJ/kg DM; $P<0.05$) and net energy for lactation at the level of production intake (NElp; 4.90 and 5.52 respectively vs. 2.68 MJ/kg DM; $P<0.05$) compared with RBP3. The RBP2 had higher *in vitro* cumulative gas production at 24 h of incubation, estimated NEl, ME and organic matter digestibility compared with RBP1 and RBP3 ($P<0.01$). Current results indicate that the RBPs could be considered as alternative feeds in ruminants feeding during dry periods.

Keywords: energy contents; *in vitro* gas production; raisin by-products; ruminants

To cite this article: Yari M, M Manafi, M Hedayati, S Khalaji, S Valinejad, R Valizadeh and A Hosseini-Ghaffari, 2015. Prediction of energy contents and potential nutrient supply of raisin by-products for ruminants using National Research Council feeding system and *in vitro* gas production method. Res. Opin. Anim. Vet. Sci., 5(6): 284-289.

Introduction

Grapevine is an important agricultural product due to its numerous usages worldwide (Ali et al., 2010). Sun dried raisin is produced from grapevine during post-harvest processing. Several raisin by-products (RBPs) consist of skin and pedicle of berries, un-ripped berries with their pedicles, stalks, rachises and pedicles of grapevines are produced during raisin production in many countries (Yari et al., 2015). During raisin production, different by-products can be produced where these by-products may be used as feed alternatives for ruminant animals during the dry periods in semi-arid climate condition. However, the nutritive value of these by-products are seldom known because of different grapevine cluster varieties used for raisin

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production but reported to have anti-nutritional factors (Valizadeh and Sobhanirad, 2009; Besharati and Taghizadeh, 2011; Yari et al., 2015).

According to FAO (2012), Iran is ranked 3rd and 9th in raisin and grapevine production capacity respectively, though considerable amount of grapevine by-products are lost in the process. Annual production of sun dried raisin in Iran is approximatly 2.87 million tons (Alipour and Rouzbehan, 2007; Besharati and Taghizadeh, 2011; Saremi et al., 2014) of which one fourth is produced in Malayer Town (Hamedan Province) from white Sultana grapevine (*Vitis vinifera L.*; IMA, 2009).

Previous investigations demonstrated that RBPs have low nutrient availability for ruminants (Abel and Icking, 1984; Yari et al., 2015) and reduced *in vivo* digestibility and performance in sheep (Abel and Icking, 1984; Tabatabaei et al., 1992; Lu and Yeap-Foo, 1999; Baumgartel et al., 2007; Moghaddam et al., 2013; Saremi et al., 2014) due to their higher phenol compounds. Application of either polyethylene glycol or polyvinyl-pyrrolidone to RBPs can improve nutrient availability for ruminant (Besharati and Taghizadeh, 2011). However, due to variation in grapevine cluster varieties used to make raisin and different RBPs produced in terminal factories, different combinations of RBPs may result in different nutrient contents and availability in ruminant feeding.

The hypothesis of this study was that energy value and potential of nutrient supply of different RBPs may not be similar in ruminants feeding. Therefore, the objectives of this study were to determine the energy contents and potential nutrient supply of RBPs for ruminants using National Research Council (NRC, 1996 and 2001) feeding system and *in vitro* gas production method.

Materials and Methods

Raisin by-products sources

Different RBPs samples were collected from two factories (Malayer Town, Hamedan province, Iran; 34°20′N, 48°45′E) as experimental replicates for chemical composition analysis, predicted energy contents by NRC, *in vitro* gas production fermentation kinetics measurements and predicted potential nutrient supply from gas production data in which the factories were considered as random blocks. These two factories had same processing methods to package raisin for export (Yari et al., 2015). Therefore, in the current study, there were three RBPs with two blocks which resulted in six different RBPs based on a randomized complete blocks design.

Predicted energy contents by National Research Council feeding system

Energy contents were predicted using chemical composition according to NRC (1996 and 2001). Chemical composition of RBPs was reported in Yari et al. (2015) in detail. Total digestible nutrient (TDN) was calculated using total digestible neutral detergent fiber (NDF), crude protein (CP), fatty acid, and non-fiber carbohydrate (NFC) according to NRC (2001). Net energy for maintenance (NE_m) and growth (NE_g) and metabolizable energy (ME) were calculated according to NRC (1996) and net energy for lactation at the level of production intake (NE_{lp}) was calculated according to NRC (2001).

In vitro gas production and predicted potential nutrient supply by developed regression equations

The semi-automated gas production procedure was conducted according to previous studies (Theodorou et al., 1994; Rogerio et al., 1999) and buffered rumen fluid prepared according to Menke and Steinglass (1988).

Rumen fluid was collected before the morning feeding from four ruminally fistulated steers (482.5 ± 22.5 kg, body weight), according to Iranian Council on Animal Care guidelines (ICAC, 1995). Ruminal content was immediately strained through four layers of cheese cloth to eliminate large feed particles and transferred to the laboratory in a pre-warmed thermos. The steers were fed 9 kg DM/day (in g/kg DM; total mixed ration with 556 g barley silage, 300 g alfalfa hay, and 144 g dairy cow concentrate) twice daily in equal portions at the experimental farm of the Ferdowsi University of Mashhad (Mashhad, Iran) as described by Yari et al. (2014).

Three vials (125 ml) per RBP sample were used to investigate *in vitro* gas production characteristics of the three by-products collected from two independent factories. Three vials with buffered rumen medium without sample were incubated to correct for gas release from the inoculums. Accumulated head-space gas pressure measurements were made using a pressure transducer (Razi Instruments, Mashhad, Iran). During the stages of this trial, it was assumed that the relationship stated in Boyle's Gas Law could be used to predict head-space gas volume (Gp) from pressure measurements according to the $GP=(Vh/Pa)\times Pt$ equation, where Vh represents head-space volume (95 ml), Pa represent atmospheric pressure (14.692 psi; Meteorological Office, Mashhad, Iran), and Pt represents pressure transducer reading (psi) (Rogerio et al., 1999; Theodorou et al., 1994). Readings were taken at 0, 2, 4, 8, 12, 20, 24, 48, 72 and 96 h after the start of incubation. Gas production incubation was carried out into two runs. The rate and extent of gas production were determined for each RBP by fitting gas production data to the nonlinear equation $Y=b(1-e^{-ct})$ (Ørskov and Mcdonald, 1979), where Y is the volume of gas produced at time t, b is the asymptotic gas production, and c is the fractional rate of gas production.

Parameters b and c were calculated using the NLIN (nonlinear) procedure of SAS using iterative least-squares regression (Gauess-Newton method) (SAS, 2003).

Previously developed linear regression equations based on gas production and chemical composition data were used to estimate the potential nutrient supply of RBPs. Organic matter digestibility (OMD), ME and NE_l was estimated using the cumulative volume of gas production at 24 h of incubation and the CP, fat and ash chemical composition of RBPs as described by Menke and Steingas (1988). Application of gas production and chemical composition data to predict feed OMD, ME and NE_l values were standardized and validated using data from 400 *in vivo* digestibility studies with corresponding *in vitro* gas production (Table 1) (Menke and Steingas, 1988). The *in vitro* true digestibility of dry matter (IVTD) and total volatile fatty acid (TVFA) was estimated using the cumulative volume of gas production at 24 h of incubation and the CP, NFC, fat and aNDF contents of RBPs as described by Getachew et al. (2004) (Table 1).

Statistical analysis

Data of current study was analyzed using PROC MIXED of SAS 9.2 (SAS, 2003) with the following statistical model:

$$Y_{ij}=\mu+T_i+B_j+e_{ij}$$

Where Y_{ij} is the observation of the dependent variable ij; μ is the fixed effect of sample mean for the variable; T_i is the fixed effect of RBPs (i=3; RBP1, RBP2 and RBP3); B_j is the random effect of block (k= 2) and e_{ij} is the random error associated with the observation ij. For gas production data analysis, the above statistical model was used and the effect of run included in the model as a random effect. For all analysis, experimental replicates were block for RBP (n=2). The Fisher's protected least significant difference test was used for multiple treatment comparisons using the LSMEAN statement of SAS. For the different statistical tests, significance was declared at $P\leq0.05$ and trends were considered at $0.05 < P < 0.10$.

Results

Predicted energy contents by national research council feeding system

The RBP1 and RBP2 treatments had greater ($P<0.05$) NRC predicted energy values of NElp, ME, NE_m and NE_g than RBP3 treatment (Table 2). The estimated energy values of NRC (2001) for NElp and NRC (1996) for ME, NEm and NEg were greater ($P<0.05$) for RBP1 and RBP2 than in RBP3.

In vitro gas production and predicted potential nutrient supply by developed regression equations

The *in vitro* cumulative volume of gas production at time of 24 h of incubation, and *in vitro* predicted nutrient supply of IVTD, ME, NE, OMD and TVFA were greater for RBP2 than for RBP1 and RBP3 ($P<0.01$; Table 3). By plotting the volume of gas production as ml/g DM, at different time of *in vitro* incubation, RBP2 had higher gas volume than both RBP1 and RBP3 (Fig.1).

Discussion

Predicted energy contents using NRC

The main difference between current RBPs and previously reported include cultivar, proportion of berry skin, quality of raisin, stalk and rachis which should be taken into consideration when discussing current results. The energy contents predicted by summative approaches reported in NRC using chemical composition. The higher energy values estimated for RBP1 and RBP2 compared to RBP3 might be due to their higher NFC, and lower aNDF and lignin (sa) contents (Yari et al., 2015). Based on those summative approaches, NFC has higher coefficient for digestibility compared with aNDF (NRC, 2001). The tabular values of NRC (2001) for the energy content of NEl_{3x}, ME_{3x}, NEm and NEg_{3x} (as MJ/kg DM) for alfalfa hay were 4.98, 8.20, 5.31, and 2.93 and for wheat straw were 3.43, 6.02, 3.47, and 1.21 respectively (NRC, 2001). Current NRC predicted energy contents for RBP1 and RBP2 are comparable with those reported by NRC 2001 for alfalfa hay at early flower stage of growth and

Table 1: Linear regression equations used to predict potential nutrient supply of different raisin by-products from gas production and chemical composition in ruminants

Predicted parameter[a]	Linear regression equations
In vitro true digestibility of dry matter (IVTD; g/kg)	IVTD=0.31 +0.0057×gas +0.0006×CP +0.00022×NFC +0.00096×fat
Organic matter digestibility (OMD; g/kg)	OMD=14.88+0.8893×gas +0.0448×CP+0.0651×ash
Total volatile fatty acid (TVFA; mM/l)	TVFA=1.84+0.56×gas+0.016×CP+0.005×aNDF
Metabolizable energy (ME; Mj/kg DM)	ME=2.2+0.1357×gas+0.0057×CP+0.0002859×CP×CP
Net energy for lactation (NEl; Mj/kg DM)	NEl=-0.22+0.1062×gas+0.0048×CP+0.0132×fat

[a]Gas, cumulative gas from 0 to 24 h (ml/0.2 g DM); CP, crude protein; NFC, non-fiber carbohydrate calculated based on NRC 2001 dairy (NRC, 2001) as NFC(g/kg DM)=1000-(CP+fat+aNDF+ash); fat, ether extract; aNDF, neutral detergent fiber.

Table 2: Predicted energy values of raisins by-products using NRC beef 1996 and NRC 2001 dairy program

Item		Raisin by-products (RBP)[d]			SEMD	*P*-value
		RBP1	RBP2	RBP3		
NRC dairy 2001 energy values (MJ/kg DM)[e]						
	NElp	4.90[a]	5.52[a]	2.68[b]	0.593	0.05
NRC beef 1996 energy values (MJ/kg DM)						
	ME	11.48[a]	9.45[a]	5.99[b]	0.735	0.05
	NE_m	7.70[a]	5.94[a]	2.51[b]	0.722	0.05
	NE_g	5.06[a]	3.47[a]	0.29[b]	0.677	0.05

[d]Raisins by-products were 1) some outer layer of flesh and skin and pedicle of berries (RBP1); 2) rejected raisins mostly un-ripped berries with their pedicles (RBP2), and 3) stalks, rachises and pedicles of grapevines; Means with different superscript letters (a, b and c) within the same row differ ($P < 0.05$); SED, standard error of difference; [e]Net energy lactation at production level of intake (NE_{lp}) was calculated according to NRC dairy 2001 and metabolizable energy (ME), net energy for maintenance (NE_m) and growth (NE_g) were calculated according to NRC beef 1996.

Table 3: *In vitro* gas production kinetics and predicted nutrient supply of raisin by-products

Item	Raisin by-products (RBP)[d]			SED	*P*-value
	RBP1	RBP2	RBP3		
Gas production characteristics[e]					
b (ml/g DM)	225.2	253.7	224.8	10.4430	0.28
c (/h)	0.1182	0.1375	0.0991	0.0124	0.29
Gas 24 h (ml/0.2 g DM)	39.93[b]	45.60[a]	38.59[b]	0.6190	<0.01
Predicted potential of nutrient supply of raisin by products using gas production kinetics					
IVTD (g/kg)	560.9[b]	589.3[a]	543.5[b]	3.480	0.01
ME (Mj/kg DM)	7.65[b]	8.43[a]	7.50[b]	0.085	<0.01
NEl (Mj/kg DM)	4.09[b]	4.69[a]	3.94[b]	0.067	0.01
OMD (g/kg)	507.8[b]	560.47[a]	501.0[b]	5.340	<0.01
TVFA (mM/l)	24.34[b]	27.59[a]	23.85[b]	0.367	0.01

[d]Raisins by-products were 1) some outer layer of flesh and skin, pedicle of berries (RBP1); 2) rejected raisins mostly un-ripped berries with their pedicles (RBP2), and 3) stalks, rachises and pedicles of grapevines; means with different superscript letters (a, b and c) within the same row differ ($P<0.05$); SED, standard error of difference; [e]b, potential of gas production; c, constant rate of gas production calculated by Orskov and Mcdonald (1987) model; Gas 24, cumulative gas from 0 to 24 h of incubation; [f]Predicted potential of nutrient supply were IVTD, in vitro true digestibility of dry matter; ME, metabolizable energy; NEl, net energy for lactation; OMD, organic matter digestibility and TVFA, total volatile fatty acids.

estimated the energy contents of RBP3 are comparable with those reported for wheat straw. These results indicate that these RBPs could be considered as an alternative feedstuffs during the shortage of common feeds. However, more researches and *in vivo* studies are required to find out their impacts on animal health and performance.

In vitro gas production kinetics and estimated energy contents

There is limited information on *in vitro* gas production kinetics of RBPs and its estimated energy contents. The total phenolic compounds and total tannins of RBP have been reported (Yari et al., 2015) to be 36.6–87.4 g/kg DM and 34.6–75.8 g/kg DM respectively. Tannins have been found to reduce gas or methane production or fermentation parameters in the rumen (Tavendale et al., 2005; Tefera et al., 2008). Higher accumulative gas during the incubation of RBP2 especially during first 24 h may be due to its lower total phenol and total tannin (Yari et al., 2015), which consequently resulted to increased predicted potential nutrient supply compared with two other RBPs. These results are in consistence with the findings of Longland et al. (1995) who reported an inverse relationship between gas accumulation and tannin content of feed samples. Feeding tannin containing plants can adversely affect ruminal protein degradation, the microbial and enzyme activities and prevent excessive ruminal gas formation (Waghorn, 2003; Wina et al., 2004). It seems that total tannin contents of RBP2 in combination with its other chemical composition such as NFC and aNDF resulted in better fermentation profile and consequently higher potential of nutrient supply in ruminant. Higher NFC with lower total tannin may result in better fermentation of NDF in gas production vials (Menke and Steingas, 1988; Getachew et al., 2004).

Addition of polyethylene glycol to tannin-containing feeds increased *in vitro* gas and short chain fatty acids production, and *in vitro* degradation of N (Besharati and Taghizadeh, 2011). Tannins also have effects on ruminal fermentation of carbohydrates, particularly hemicellulose, cellulose, starch, and pectin compounds (Hagerman et al., 1992).

In vivo studies are required in future to find out the RBPs effects on ruminal fermentation profiles and animal health in different situations and in different combination with other diets ingredients.

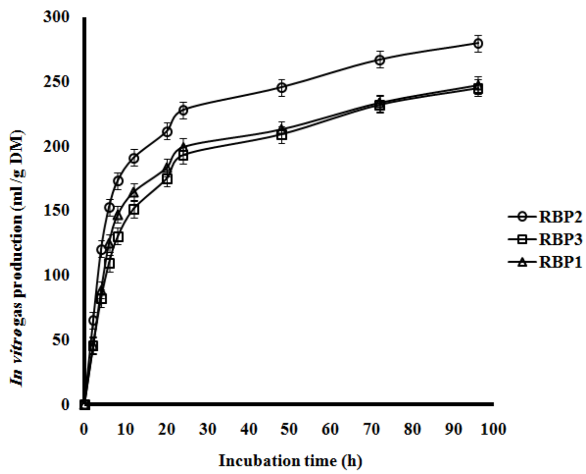

Fig. 1: Pattern of *in vitro* gas production fermentation of raisin by-products (RW). Raisins by-products were 1) some outer layer of flesh and skin and pedicle of berries (RBP1); 2) rejected raisins mostly un-ripped berries with their pedicles (RBP2), and 3) stalks, rachises and pedicles of grapevines.

Conclusion

Among the three RBPs, RBP1 and RBP2 had higher energy contents while RBP2 had better fermentation pattern and nutrient supply. The RBPs could be considered as an alternative feed ingredient to replace partially the forage portion in the diet of ruminants under the semi-arid climate condition. Current data for energy contents and potential nutrient supply of different raisin by products could be used for diet formulation.

Acknowledgment

The authors gratefully thank the Malayer University for financial support of this project.

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