Iranian Journal of Applied Animal Science

Effect of Graded Substitution of Barley Grain with Raisin Waste in Diet Contained Low Quality Forage on Growth Performance, Blood Metabolites and Nutrient Digestibility of Growing Ram Lamb

Research Article

M. Ahmadi[1], M. Yari[1*] and M. Hedayati[1]

[1] Department of Animal Science, College of Agriculture, Malayer University, Malayer, Iran

Received on: 1 Sep 2021
Revised on: 15 Mar 2022
Accepted on: 31 Mar 2022
Online Published on: Sep 2022

*Correspondence E-mail: m.yari@malayeru.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

Raisin waste (RW) is produced during grapevine processing after harvest for raisin production. The aim of current study was to substitute the barley grain with RW in diet containing cereal straw as the only forage source in growing ram lambs feeding. Ground barley was substituted with RW at the rate of 9and 18% of diet (dry matter (DM) basis) in iso-nitrogesnous and iso-energetic diets. Twelve growing ram lames (first body weight of 26±2 kg) used in 3 × 3 replicated Latin square with three experimental periods. At each period of experiment, 6 pens of lambs (each 2 lambs placed in each pen) were randomly assigned to 3 treatments (2 pens for each treatment). Raisin waste contained (% DM) 78.4 DM, 5.4 ash, 3.7 crude protein (CP), 10.6 ether extract, 18.5 acid detergent fiber (ADF), 1.4 total tannin, 2.2 total phenol and 0.8 non-tannin phenolic. Dietary substitution of ground barley with RW did not influence lamb final body weight (32.9, 32.2 and 32.1 kg respectively for three treatments), average daily gain, feed intake (1060.1, 960.2 and 920.3 g respectively) and apparent total tract nutrient digestibility including DM, organic matter, CP and ADF (P>0.05). Blood glucose, red blood cells and white blood cells count were similar among treatments (P>0.05). Blood urea decreased in lambs fed with diets contained RW (P<0.05). Blood total protein and triglycerides increased mostly at the level of 9% of substitution in lambs (P<0.05) compared with other level of substitution. In conclusion, in diets contained cereal straw as the only forage source, up to 18% of ground barley could be replaced by RW without detrimental effects on growth performance, nutrient digestibility and blood measures in growing ram lambs.

KEY WORDS barley grain, cereal straw, lambs, performance, raisin waste, tannin.

INTRODUCTION

Sheep population in Iran has been reported to be ~ 47 millions in which is almost 45 percentage of total livestock population (Vice President for Livestock Production, 2017; SCI, 2021). In developing countries, small ruminants usually consume poor quality roughages such as straws, stovers and senescent native pastures as a major part of their diet, particularly during the dry season when higher quality forages are in short supply. However, intake and digestibility of these poor quality roughages can be improved by supplementing with small amounts of higher quality material. Cereal grains have been become an important component of the diet of ruminants in many countries because they have a high energy value which supports high milk production or rapid growth rates. Barley (*Hordeum vulgare*) is one of the major cereal crops grown in terms of cultivated area. Barley is widely used in growing ram lamb feeding in Iran as a primary source of dietary energy (Gholizadeh *et al.* 2014; Ghorbani and Hadj-Hussaini, 2002; Gholizadeh *et al.*

2021). However, more frequent and severer droughts have caused water scarcity for cereal production in Iran (Abbasi *et al*. 2008; Gholizadeh *et al*. 2014; Gholizadeh *et al*. 2021). Iran is one of the major grapevine growers in the world with 215000 ha producing 2.2 million tons of fruit and Malayer is the leading grapevine growing areas (Hamedan province) of Iran, with about 11200 ha grapevines (Karimi and Ershadi, 2014; FAOSTAT, 2017). 'Sultana' (synonym White Kishmish) is one of the main grapevine cultivars planted in Malayer, which produces seedless fruit and raisin that are mainly exported as raisin or locally sold fresh for human consumption (Karimi and Ershadi, 2014; FAOSTAT, 2017). Sun dried raisin is produced from grapevine during post-harvest processing. Raisin waste (RW) consist of skin and pedicle of berries, un-ripped berries with their pedicles, stalks, rachises and pedicles of grapevines are produced during raisin production (Moghaddam *et al*. 2013; Saremi *et al*. 2014; Yari *et al*. 2015a; Yari *et al*. 2015b). The RW could be used in ruminant's feeding, but its nutritive value should be considered (Besharati and Taghizadeh, 2011; Moghaddam *et al*. 2013; Yari *et al*. 2017; Babau *et al*. 2019). The RW had low nutrient availability for ruminants (Yari *et al*. 2015a; Yari *et al*. 2015b; Yari *et al*. 2017) and reduced *in vivo* digestibility and performance in sheep (Tabatabaei *et al*. 1992; Baumgartel *et al*. 2007; Saremi *et al*. 2014) due to their higher total tannin and phenol compounds. The weather and soil condition of growing of grapevine and the variety could influence on the RW nutrient profile and availability in ruminant feeding (Yari *et al*. 2017; Babau *et al*. 2019; Iaani and Martino, 2020). The study hypothesis was that ground barley grain in diet containing cereal straw as the only forage source could be replaced by RW in growing lamb feeding. Therefore, the objective of current study was to investigate the effects of substitution of RW at the different levels with ground barley grain on growth performance, blood metabolites and total tract apparent *in vivo* nutrient digestibility of growing ram lambs.

MATERIALS AND METHODS

Animal, treatments and management

Raisin waste contained, in dry matter basis (% of DM), 78.4 DM, 5.4 ash, 3.7 crude protein (CP), 10.6 ether extract (EE), 18.5 acid detergent fiber (ADF), 1.4 total tannin (TT), 2.2 total phenol (TF) and 0.8 non-tannin phenolic. Three experimental treatments were to substitute ground barley grain with RW at the rate of 9 (low) and 18% (high; DM basis). Current three experimental treatments were 1) control, 2) low raisin waste (LRW) and high raisin waste (HRW; Table 1). Current experiment was conducted at Dorud city (33.4955˚ N, 49.0632˚ E), Lorestan province, Iran in a commercial farm during summer 2019. All the animal procedures were approved by the Iranian Council of Animal Care (1995). Twelve growing ram lambs with the first body weight 26.2 ± 2.3 (BW; mean±SD), aged 5 months old, were ear tagged and vaccinated against pulpy kidney (2.5 mL/lamb) and foot and mouth disease 1ml/lamb) and agalactia (2.5 mL/lamb) from Razi Vaccine and Serum Research Institute (Tehran, Iran), treated for internal parasites using closantel 5% (RooyanDarou, Tehran, Iran) (5 mL/lamb) and external parasites (ivermectin, RooyanDarou, Tehran, Iran), shearing and hoof trimming. The lambs were randomly assigned to three treatments and housed in pens (2 m×2 m) (two lambs per each pen). Pens were separated by a wall from each other and inside the pen lambs had access to their own water drinker and feeder. The lambs were then adapted to the experimental diets and pens for 10 days before beginning the experiment to calculate DM intake and BW for including in the final statistical model as covariates.

Three experimental diets were changed among 6 pens of lambs as 3 × 3 Latin square design for three periods. In each experimental period, two pens of lambs were randomly assigned to the diets which in previous period not received. Experimental diets were formulated to meet nutrient requirement of lambs (NRC, 2007). The diets were offered to lambs to have 5% ort.

Sample collection and chemical analysis

Each period consisted of 14 days adaptation to the experimental diet and 7 days for sample collection. During sample collection period, DM intake was measured daily and BW was measured at the end of each period. Fecal samples from the bed of each pen were collected for three consecutive days and air dried and kept for further chemical analysis. Acid insoluble ash (AIA) was measured in experimental diet and fecal samples and used as an internal marker to estimate apparent total tract *in vivo* digestibility of DM, organic matter (OM), CP and ADF (Van Keulen and Young, 1977).

At the last day of each sample collection period and at the before AM feeding, blood samples collected from jugular vein by venoject tube from lambs. Blood samples separated immediately after bloods withdraw in to a tube contained EDTA for blood cell counts and other one in tube in which coagulated. Samples close to the ice were transferred to lab for further analysis. Procedures followed for blood metabolites and cells analysis were enzymatic calorimetric using photometric method for glucose (GOD-PAP) and triglyceride (GPO-PAP) assay, enzymatic for urea nitrogen (Urease GLUDH) and photometric for total protein (Biuret method). Special kits for glucose, triglyceride, total protein and urea nitrogen were from Pars Azmoon (Tehran, Iran).

Table 1 Ingredients and chemical composition (DM basis) of diet

Ingredients	Experimental diets[1]		
	Control	LRW	HRW
Wheat straw	47.0	45.0	44.0
Ground barley	35.0	27.0	18.0
Soybean meal 48%	8.0	9.0	10.0
Wheat bran	8.0	8.0	8.0
Raisin waste (RW)	0.0	9.0	18.0
Mineral and vitamin supplement[2]	0.5	0.5	0.5
Salt	0.5	0.5	0.5
Sodium bicarbonate	1.0	1.0	1.0
Chemical composition			
Dry matter	87.50	85.81	85.52
Crude protein	11.00	11.00	11.00
Metabolizable energy (Mcal/kg DM)	2.18	2.18	2.18
Net energy for gain (NEg, Mcal/kg DM)[3]	0.75	0.75	0.75
Acid detergent fiber	27.52	28.02	28.53
Total tannin	0.48	0.60	0.68
Total phenol	0.15	0.25	0.24
Non-tannin phenolic	0.32	0.35	0.43
Price (IR-Rial)	7017.51	6727.53	6487.52

[1] LRW: substitution of 9% of diet barley with raisin waste and HRW: substitution of 18% of diet barley with raisin waste.
[2] Mineral and vitamin supplement in which each kg of the supplement contained: vitamin D: 100000 IU; vitamin A: 600000 IU; vitamin E: 3000 IU; Ca: 185 g; P: 35 g; Mg: 21 g; Na: 24 g; Mn: 3 g; Cu: 0.8 g; Iron: 2 g; Zn: 4 g; I: 50 mg; Se: 14 mg and Co: 29 mg.
[3] NEg: energy values predicted by CPM dairy v3 using actual values of chemical composition of diet ingredients.

The red blood cell and white blood cell counts were performed using Sysmexkx-21N cell counter (MAN Company, Iran).

Standard procedures described by the Association of Official Analytical Chemists (AOAC, 1990) were used to determine dry matter (method 930.15), ash (AOAC method 942.05), crude protein (CP; AOAC method 984.13) and ether extract (EE; AOAC method 954.02). Acid detergent fiber (ADF) were determined according to Van Soest *et al.* (1991) with the ANKOM A200 Filter Bag Technique (Ankom Technology, Fairport, NY, USA). Acid detergent lignin (ADL) was determined by soaking the ADF filter bag residue in 72% sulphuric acid for 3 h followed by washes with water (AOAC method 973.18). For diet and fecal samples, DM, ash, CP and ADF were measured as described above (AOAC, 1990).

Total phenolics were measured using the Folin Ciocalteau method (Makkar, 2000). Total tannin was determined after adding insoluble polyvinylpyrrolidone 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.

Statistical analysis

All data were analyzed using the MIXED procedure of SAS (2003). The following model was used:

$$Y_{ijk}= \mu + T_i + P_j + P \times T_{ij} + BW_k + S_l + e_{ijkl}$$

Where:
Y_{ijk}: observation of the dependent variable.
μ: fixed effect of population mean for the variable.
T_i: fixed effect of treatment (i=3).
P_j: fixed effect of period (j=3).
$P \times T_{ij}$: fixed effect of interaction between T at the level of i and P at the level of j.
BW_k: covariate of first body weight before beginning of the experiment.
S_l: fixed effect of square.
e_{ijkl}: random error associated with the related observation.

First body weight of lambs included in the final statistical model as a covariate factor and was excluded from the model when $P > 0.10$. Tukey's multiple range test was used for multiple treatment comparisons using the LSMEAN statement of SAS 9.2 (SAS, 2003) with letter grouping obtained using the SAS pdmix 800 macro (Saxton,1998). For the different statistical tests, significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$, unless otherwise stated.

RESULTS AND DISCUSSION

Growth performance and nutrient intake of lambs fed with experimental diets are shown in Table 2. Body weight, average daily gain, FCR, BW change, average DM intake, ADF intake, OM intake and CP intake of lambs across different period of study were similar among treatments (P>0.05).

Table 2 Effect of graded substitution of diet ground barley with raisin waste on growing ram lamb growth performance

Parameters	Treatments			SEM	Significant levels[1]		
	Control	LRW	HRW		Treatment	Period	Treatment × period
Body weight (kg)	32.9	32.2	32.1	0.33	0.46	< 0.01	0.17
Feed intake (g)	1060.1	960.2	920.3	60.38	0.40	< 0.01	< 0.01
ADG (g)	173.1	142.2	140.3	29.21	0.48	< 0.01	0.15
FCR	6.6	6.5	6.3	0.33	0.83	< 0.01	0.25
BWC (kg)	3.6	3.0	2.9	0.323	0.29	< 0.01	0.14
ADFI (g)	340.1	310.5	290.4	14.12	0.22	< 0.01	< 0.01
OMI (g)	920.3	860.4	810.1	39.32	0.27	< 0.01	< 0.01
CPI (g)	132.5	123.4	116.3	5.01	0.21	< 0.01	< 0.01

[1] Treatment × period: interaction between treatments and experimental periods.
LRW: substitution of 9% of diet ground barley with raisin waste; HRW: substitution of 18% of diet ground barley with raisin waste; ADG: average daily gain; FCR: feed conversion ratio; BWC: body weight change; ADFI: acid detergent fiber intake; OMI: organic matter intake and CPI: crude protein intake.
SEM: standard error of the means.

Coefficients of total tract apparent *in vivo* nutrient digestibility of lambs fed experimental treatments are shown in Table 3. Coefficients of DM digestibility, OM digestibility, CP digestibility and ADF digestibility were similar among lambs fed with the experimental diets (P>0.05).

Blood cell counts and metabolites of lambs fed experimental treatments are shown in Table 4. Blood glucose concentration and red blood cells and leucocytes count were similar among lambs (P>0.05). Blood triglyceride concentration was greater in lambs fed with LRW diet compared with lambs fed control diet and HRW diet (P=0.05). Lambs fed with HRW diet had greater blood triglycerides compared with lambs fed with control diet (P=0.05). Lambs fed with LRW diet had greater blood total protein compared with lambs fed control and HRW was intermediate (P<0.05). Lambs fed with control diet had greater blood urea compared with lambs fed with diets contained RW (P<0.05).

During the processing of grapevine for raisin production, different wastes are produced which they have different chemical composition, energy values and nutrient availability for ruminants (Yari *et al*. 2015a; Yari *et al*. 2015b). The RW used in current study was mainly consisted of some outer layer of flesh and skin and pedicle of berries (Yari *et al*. 2015b). Also, the RW may consist of rejected raisins mostly un-ripped berries with their pedicles, stalks, rachises and pedicles of grapevines. Therefore, chemical composition of RW is dependent on their content. The RW that contained stalks, rachises and pedicles of grapevines usually have greater structural carbohydrate, lignin, total tannin content and lower nutritive value for ruminants (Besharati and Taghizadeh, 2009; Saremi *et al*. 2014; Yari *et al*. 2015a; Yari *et al*. 2015b). Total tannin and total phenolics of current RW were close but the ADF, CP and ash were greater than those reported by Alipour and Rouzbehan (2007). Current RW had comparable ash, CP, ADF and EE but lower total tannin (1.4 *vs*. 5.4% of DM) and total phenol content (2.2 *vs*. 6.7% of DM) compared with those reported by Besharati and Taghizadeh (2009). Variety of grapevine, planting and harvesting condition and methods of processing of grapevine after harvest are main factors that could influence the chemical composition and consequently nutrient profile and availability of RW for ruminants (Karimi and Ershadi, 2014; Yari *et al*. 2015a; Yari *et al*. 2015b; Yari *et al*. 2017).

In current study, 90 g RW/kg of diet DM (LRW) and 180 g RW/kg of diet DM (HRW) were fed to growing male lambs. Growth performance and *in vivo* nutrient digestibility were similar among lambs fed with different diets. Lignin and total tannin and total phenolics content in RW have been reported as the limitation factors for its usage in ruminants diets (Alipour and Rouzbehan, 2007; Besharati and Taghizadeh, 2009; Saremi *et al*. 2014; Yari *et al*. 2017; Babau *et al*. 2019) which could reduce its nutrient availability. Saremi *et al*. (2014) reported that RW can be included in the diet of growing lambs at the level of 100 g/kg of diet DM without adversely affecting the digestibility of diet nutrients but feeding lambs with more than 200 g RW/kg of diet DM reduced protein digestibility, nitrogen retention and absorption.

Tannins bind to protein and decrease accessibility of proteins to rumen microorganisms in which it could decrease protein digestibility and nitrogen absorption and retention (Babau *et al*. 2019; Iaani and Martino, 2020). Inclusion of moderate levels of RW in the ration provides sheep industry with an inexpensive feed and reduces the environmental impact of waste disposal in the raisin production industry (Babau *et al*. 2019). In this study, current RW had calculated net energy for gain (NEg) of 1.2 (Mcal/kg DM) (Yari *et al*. 2015b) which is close to the value of 1.3 (Mcal/kg DM) NEg of ground barley (NRC, 2001). Its calculated NEg might be due to its higher non-fiber carbohydrate (NFC) content versus barley grain (76% *vs*. 60% of DM). The main component of its NFC, is simple sugars like fructose and glucose (Alipour and Ruzbehan, 2007) but in barley, the main NFC content is starch.

Table 3 Effect of graded substitution of diet ground barley with raisin waste on growing ram lamb *in vivo* apparent total tract nutrient digestibility

Parameters	Treatments			SEM	Significant levels[1]		
	Control	LRW	HRW		Treatment	Period	Treatment × period
DMD (%)	70.7	68.9	68.2	0.94	0.21	< 0.01	0.48
OMD (%)	73.1	71.5	71.3	0.65	0.18	< 0.01	0.15
CPD (%)	70.6	70.0	68.9	1.535	0.75	0.08	0.86
ADFD (%)	40.3	40.4	40.6	2.65	0.99	0.10	0.19

[1] Treatment × period: interaction between treatments and experimental periods.
LRW: substitution of 9% of diet ground barley with raisin waste; HRW: substitution of 18% of diet ground barley with raisin waste; DMD: dry matter digestibility; OMD: organic matter digestibility; CPD: crude protein digestibility and ADFD: acid detergent fiber digestibility.
SEM: standard error of the means.

Table 4 Effect of graded substitution of ground barley of diet with raisin waste on growing ram lamb blood metabolites

Parameters	Treatments			SEM	Significant levels[1]		
	Control	LRW	HRW		Treatment	Period	Treatment × period
Glucose	69.9	71.1	73.2	2.59	0.66	< 0.01	0.29
Triglyceride	17.8[c]	22.7[a]	20.3[b]	1.18	0.05	< 0.01	0.10
Total protein	6.8[b]	7.0[a]	6.9[ab]	0.13	0.04	< 0.01	0.53
Urea	16.1[a]	13.7[b]	12.4[b]	0.97	< 0.01	< 0.01	0.97
Red blood cells	5.3	5.1	5.2	0.23	0.76	< 0.01	0.72
Leucocytes	12.6	13.8	12.8	1.01	0.39	0.08	0.04

[1] Treatment × period: interaction between treatments and experimental periods.
LRW: substitution of 9% of diet ground barley with raisin waste and HRW: substitution of 18% of diet ground barley with raisin waste.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The NFC content could be used as the energy source for rumen microbes to capture more ammonia for production of microbial protein and improve the fermentation process in rumen. Also, current diets had very low total tannin and total phenol content which was not as much as high to decrease animal performance and BW gain (Babau *et al.* 2019; Iaani and Martino, 2020).

In current study, the only forage source for diets was straw. Generally, the straw has very low simple sugar content and high concentration of NDF. Diets based on straw as the only forage source, may need to be supplemented with NFC sources for more ruminal efficient fermentation (Klevenhusena and Zebeli, 2021). By addition of RW in place of barley grain, the simple sugars concentration has been increased in diets. These sugars are commonly considered to be a carbohydrate fraction that ferments faster in the rumen than starch (Oba, 2011). Synchronization between N degradation and total volatile fatty acids (VFAs) production during fermentation in rumen, determine the amount of final ammonia which would be absorb through the ruminal wall in blood which then transferred to the liver and convert to urea and would be back in blood (Oba, 2011). Excess rumen ammonia results in ammonia absorption, whereas a deficiency of rumen ammonia encourages urea recycling, and so long as ruminally degraded protein intake is in close balance with microbial protein synthesis, the need to account for urea recycling is minimal (NRC, 2007). This may have been resulted to capture more ammonia nitrogen by ruminal microbes and to convert to microbial protein and therefore decreased the blood urea nitrogen in lambs fed with RW diets. Increased microbial protein from the ruminal degradable protein might have been increased metabolizable protein and increased total protein in blood of lambs fed with LRW diet.

Changes in blood urea and total protein in response to addition of RW in diets in lambs might also be due to the tannin and phenol content of RW. Tannin is known for its anti-nutritional properties due to its detrimental effects on feed intake, rumen microorganisms, nutrient utilization, and production performance of ruminant livestock, particularly when present at a high concentration in the diet (>50 g/kg DM). However, when present at a low to moderate level, tannin may provide beneficial effects to modulate ruminant performance, health, and environmental sustainability (Rizki Yanza *et al.* 2021). Research has shown that diets contained condensed tannin (>50 g/kg DM) can disrupt ruminal microbial digestion (Kelln *et al.* 2021). Current RW had lower total tannin content compared with recommended level (1.4 *vs.* 50 g/kg). In a recently published meta-analysis, Rizki Yanza *et al.* (2021) reported that the plasma urea-N was decreased by a quadratic response when the supplementation of tannin extract increased and tended to be significantly influenced by the type of tannin extract. Although the albumin was not influenced by the tannin extract concentration, the type of tannin tended to affect the albumin concentration in the blood plasma. The decrease in ammonia (NH_3) concentration also showed an obvious relationship with the increased level of tannin. The feed particles might form fibre–tannin and protein–tannin complex bonds in which might be difficult to degrade by proteolytic bacteria. Thus, protein and amino acids might be protected by tannin to pass rumen fermentation and could befavorable because this would increase protein absorption in the small intestine, which in turn increases N use efficiency (Kelln *et al*. 2021; Rizki Yanza *et al*. 2021).

Lambs fed with RW had greater blood triglycerides compared with lambs fed control diet. During processing of grapevine after harvesting to convert it to raisin, olive oil and potassium bicarbonate are added as a chemical solution to grapevine in which this would increase the ash and ether extract content of RW (Yari *et al*. 2017). It seems that substitution of diet barley grain by RW had increased the ether extract concentration of diet and this has been caused to greater blood triglycerides in those lambs fed with RW diets. The price of RW used in this study was lower than barley grain price and its including in diet, decreased total cost of diet (Table 1).

CONCLUSION

Total tannin and phenol concentration of current raisin waste was < 50 g/kg of dry matter. Substitution of ground barley with raisin waste at 9 and 18% of dietary inclusion rate did not had detrimental effects on growth performance, blood measures and *in vivo* apparent total tract nutrient digestibility in growing lamb feeding when straw was the only dietary forage source. Using higher inclusion rate in growing lamb diet need further research in future.

ACKNOWLEDGEMENT

Authors would like to thank the Malayer University to partially financial support of current study.

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