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Effect of different levels of raisin waste on performance, nutrients digestibility and protozoal population of Mehraban growing lambs

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Abstract

The aim of this study was to assess the effects of different inclusion levels of raisin waste (RW) in the diet on the animal performance and ruminal fermentation parameters of growing lambs. Four levels of RW inclusion (*i.e.*, R_0, R_1, R_2 and R_3 for 0, 100, 200 and 300 g RW kg^{-1} dry matter of diet, respectively) were tested. The experimental diets were fed to 24 male lambs (six months old) and six animals were allocated to each treatment. In the first experiment, effects of different levels of RW on the animals' performance, some rumen parameters and protozoa populations were studied. In the second experiment, the apparent total tract digestibility of diets and nitrogen balance were measured. The highest final body weights were observed for the R_2 and R_3 diets. The R_3 diet had the lowest dry matter intake (1156 *vs.* 1303 g day^{-1} for R_3 and R_0, respectively) and feed conversion rate (6.4 *vs.* 8.7 for R_3 and R_0, respectively). Total number of protozoa increased with the addition of RW, but *Epidinium* spp. completely disappeared with the R_3 diet. Inclusion of RW at levels higher than 200 g RW kg^{-1} DM of diet significantly reduced crude protein ($p = 0.042$) and neutral detergent fiber digestibility ($p = 0.049$). Our findings showed that RW could be included in the diets of growing lambs up to 200 g kg^{-1} DM without compromising their production performance.

Additional key words: rumen fermentation; ciliates; sheep; live weight gain; agro-industrial by-products.

Introduction

Iran is located in a semi-arid zone, and sometimes the livestock holders face to shortage of feed supply due to drought. One way to overcome this scarcity is to feed animals with locally available feed resources and agro-industrial by-products which cannot be consumed by humans. Raisin waste (RW) is a co-product of raisin production which is left after sorting and packing of raisins, and composed of rejected raisins, rachis, peduncles and pedicles.

The RW, like some other by-products, contains tannins (Besharati & Taghizadeh, 2011). Tannins are categorized as condensed and hydrolysable tannins which have positive and negative effects in ruminant nutrition (Makkar, 2003a). Within the latter, Priolo *et al.* (2000) found that feeding the lambs with carob pulp decreased the weight gain compared to those fed with conventional maize based diet. The negative effects of tannins are attributed to their capacity to bind to proteins followed by precipitation in the rumen (Jones & Mangan, 1977), reduced rumen protein degradation and fractional absorption of amino acids reaching to the small intestine (McNabb *et al.*, 1996). These consequences result in low digestibility and low voluntary feed intake (Reed, 1995). Tannins also exert beneficial effects, such as protection of dietary proteins from being degraded in rumen, preventing bloat, defaunating activity and anti-parasitic effects (Min *et al.*, 2003). In an experiment, Wang *et al.* (1996) observed that grazing sheep on *Lotus corniculatus* increased live weight gain and carcass weight compared to those supplemented

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Abbreviations used: CT (condensed tannins); D_{144} (potential degradable dry matter); DM (dry matter); E (extent of degradation); FCR (feed conversion ratio); FLW (final live weight); GP (gas production); L (linear); LW (live weight); LWG (live weight gain); OM (organic matter); q (quadratic); RW (raisin wastes); TP (total phenolic); TT (total tannins); UN (urinary nitrogen); VFA (total volatile fatty acids).

with polyethylene glycol which binds tannins and deactivates them. These varying responses are related to the type, structure, amount and source of tannins (Makkar, 2003a). Using tannin containing feeds in animal ration may improve the nutrient utilization and productivity of ruminants. In an experiment, inclusion of red quebracho (*Schinopsis quebracho-Colorado* (Schlecht) and Barkley & T. Meyer) tannin in the diet increased the live weight gain (LWG) of lambs in comparison to control group (Dawson *et al.,* 2010). To our best knowledge, no information is available on the effect of RW and its tannins on performance of growing lambs and rumen fermentation parameters. Therefore, in the current study we assessed the effects of different levels of RW in the diet of growing lambs on their production performance, protozoal population and nitrogen retention.

Material and methods

Experiment I

Twenty four male Mehraban lambs were used in this trial. Animals were born in a same farm and weaned at 60 days. The lambs had free access to a mixture of commercial concentrate and chopped alfalfa (*Medicago sativa* L.) hay from day 15 of age until the beginning of experiment when they were 5 to 6 months old. Then the lambs (average weight 30 ± 3.26 kg) were placed in individual pens and randomly assigned to one of the four experimental treatments (Table 1): R_0 (control diet), R_1 (containing 100 g of RW kg^{-1} DM of diet), R_2 (containing 200 g of RW kg^{-1} DM of diet) and R_3 (containing 300 g of RW kg^{-1} DM of diet). The lambs were fed to meet their daily nutrient requirements (NRC, 1985) and were adapted to the experimental diets over a period of 14 days. The experiment lasted 60 days. Lambs were fed ad libitum twice daily at 08:00 and 16:00 hours to approximately a 10% refusal level, and had free access to drinking water.

Live weight gain (LWG) was calculated as the change in live weights (LW) between two consecutive measurements at the beginning and day 60 of experiment divided by the number of days between the measurements. Feed conversion ratio (FCR) was calculated as the average individual feed intake (g) per g of weight gain. The refusals were removed and weighed daily to determine voluntary DM intake. Feed and feed refusal samples from lambs within each treatment group were composited and stored at 4°C.

On day 50 of the experimental period, samples of rumen fluid were taken from each sheep before morning feeding using a stomach tube. The samples were immediately filtered through four layers of cheese cloth and stored at –30°C for pending ammonia and total volatile fatty acids analyses (VFA). Additionally, on day 55 samples of rumen fluid were obtained three hours after morning feeding to count total and different genera of rumen ciliated protozoa. To preserve the protozoa, 50 mL of rumen fluid were added to bottles containing the same volume of formalin.

To determine the fermentation kinetics of experimental diets, an *in vitro* gas production (GP) trial was carried out using 100-mL glass syringes (Menke & Steingass, 1988). In brief, rumen fluid was collected before morning feeding from three ruminally fistulated Mehraban sheep fed twice daily a diet containing 700 g kg^{-1} of alfalfa hay and 300 g kg^{-1} of a commercial concentrate (dry matter basis). The rumen fluid was filtered through four layers of cheese cloth, transferred to a pre-warmed thermos flask and pooled prior to incubations. All the operations were carried out under CO_2 flow. Ground samples (200 mg) of experimental diets were placed in the syringes. The syringes were filled with 30 mL of buffered-rumen fluid (Makkar, 2003b), and incubated in water bath at 39°C. The volume of gas produced was recorded at 1, 4, 7, 10, 13, 16, 19.5, 23, 26, 29, 32, 36, 40, 45, 50, 55, 61, 96.5, 80, 96, 120 and 144 h after incubation. At the end of incubation period, the contents of each syringe were filtered using sintered glass crucibles (coarse porosity no. 1, pore size 100-160 μm) under vacuum and oven-dried at 100°C for 48 h to estimate the potentially degradable DM (D_{144}, g kg^{-1} DM).

Gas production profiles were fitted to the exponential model proposed by France *et al*. (2000):

$$G = A[1 - e^{-c(t-L)}]$$

where G (mL g^{-1} OM) denotes the cumulative gas production at time t; A (mL g^{-1} OM) is the asymptotic of gas production; c (h^{-1}) is the fractional rate of gas production and L (h) is the lag time. According to France *et al*. (2000), the extent of degradation in the rumen (E, g kg^{-1} DM) at any rate of passage (k, h^{-1}) can be estimated as:

$$E = \frac{c \times D_{144} \times e^{-kL}}{c + k}$$

To calculate E, a rate of passage of 0.03 h^{-1} (considered to be characteristic of sheep fed on a forage diet at

Table 1. Ingredient and chemical composition and *in vitro* gas production kinetics of experimental diets fed to Mehraban growing lambs

	Experimental diets[1]			
	R_0	R_1	R_2	R_3
Ingredients (g kg⁻¹ DM)				
Alfalfa hay	540	470	370	320
Barley grain	400	370	370	320
Soybean meal	50	50	50	40
Raisin wastes[2]	0	100	200	300
Mineral and vitamin supplements	10	10	10	10
Chemical composition (g kg⁻¹ DM)[3]				
DM	912	912	920	931
OM	935	915	903	924
CP	155	147	138	129
EE	15.5	15.7	15.5	15.5
aNDF	242	231	221	214
NFE	526	542	562	576
Ash	62	64	63	65
ME	2.5	2.5	2.6	2.6
In vitro *gas production kinetics*[4]				
A	292	263	274	289
C	0.13	0.12	0.13	0.13
D_{144}	0.52	0.53	0.53	0.54
E	0.34	0.34	0.34	0.34

[1] R_0, R_1, R_2 and R_3 = 0, 100, 200 and 300 g raisin wastes per kg DM of diet, respectively. [2] The chemical compositions of raisin wastes were (g kg^{-1} DM): DM = 917 g per kg fresh weight, OM = 93, CP = 71, EE = 16.4, NDF = 170, NFE = 667, ash = 75, ME = 2.5, total phenolics = 28.8, total tannins = 25.6, condensed tannin = 8.4. [3] DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, aNDF = neutral detergent fiber analyzed by heat stable β-amylase , NFE = nitrogen free extract, ME = metabolizable energy estimated using *in vitro* gas production (MJ kg^{-1} DM) based on an equation developed by Menke & Steingass (1988). [4] A = asymptotic gas production (mL g^{-1} OM), c = rate of gas production (h^{-1}), D_{144} = disappearance of dry matter after 144 h (g kg^{-1} DM), E = effective dry matter degradability (g kg^{-1} DM). No difference ($p > 0.05$) among diets were detected in terms of i*n vitro* parameters.

maintenance level) was used (Table 1). Three incubations were completed on 3 consecutive weeks.

Experiment II

At the end of the Experiment I, four animals in each group were randomly selected and were transferred to the individual metabolic cages to measure nutrients digestibility and nitrogen balance. After a 10-day adjustment period, faeces were collected during 7 days and daily aliquot (150 g kg^{-1}) was stored at –4°C. Faecal samples were dried to a constant weight in a forced air oven at 60°C and stored before grinding for chemical analyses.

During the collection period, urine was collected in the buckets containing 100 mL of sulphuric acid (H_2SO_4; 10% v/v). Urine samples (100 mL) were stored at –4°C. At the end of the collection period, an aliquot of the composited urine from each sheep was sampled and analyzed for total urinary nitrogen (UN). Endogenous N was calculated from the difference between faecal N and insoluble faecal N in neutral detergent solution. Neutral detergent insoluble N was measured by analyzing faecal NDF residues for CP (Mass *et al*., 1999).

Chemical analysis

Feeds, refusals and faecal samples were dried at 60°C and ground to pass a 1-mm screen using a Cyclotech Mill (Tecator, Höganäs, Sweden) and were analyzed using standard methods of AOAC (1995) for DM, nitrogen (N) and ash. Neutral detergent fiber (NDF) in samples was estimated according to Van Soest *et al.*

(1991) with addition of α-amylase but without sodium sulphite and results were expressed with residual ash. Total phenolics (TP), total tannins (TT) and condensed tannins (CT) in RW were calculated as described by Makkar (2003b). The concentration of ammonia in rumen fluid was determined using the phenol-hypochlorite method (Broderick & Kang, 1980). The concentration of VFA was measured according to steam distillation method described by Markham (1942). Total numbers and generic composition of ciliate protozoa were determined according to the procedures of Dehority (1993).

Statistical analysis

Data of animal performance were analyzed using the GLM procedure of SAS (version, 9.1) to determine least square means and the significance of differences among the treatments. For evaluating the effects of levels of RW on the measured parameters linear (L) and quadratic (Q) contrasts (for trend comparison) and control *vs*. average of other treatments contrast (for class comparison) were used. The model used was:

$$Y_{ij} = \mu + L_i + (\beta \times IW_j) + e_{ij}$$

where Y_{ij} is the dependent variable (feed intake and digestibility, LW and efficiency), μ is the overall mean, L_i is the effect of level of RW, IW_j is the effect of initial weight, β is the regression coefficient estimating the effects of initial LW (covariate used for all response parameters in first experiment) and e_{ij} the residual error.

For GP data the total number of observations was 4 (Level of RW) × 3 (runs) × 4 (reps) = 48 observations, and were subjected to analysis of variance using the following model:

$$Y_{ijk} = \mu + L_i + R_j + LR_{ijk} + e_{ijk}$$

where Y_{ijk} is the observation, μ is the overall mean for each parameter, L_i is the effect of level of RW, R_j is the runs (periods) of incubation, LR_{ijk} is the interaction between levels of RW and periods of incubation, and e_{ijk} is the residual error. Because the protozoal population was not normally distributed among the animals and treatments, the data were log-transformed and analyzed using the GLM procedure of SAS 9.1. Pearson linear correlation coefficients were computed between response variables using the CORR procedure of SAS 9.1.

Results

Experiment I

The effect of different levels of RW on performance of lambs is shown in Table 2. Inclusion of RW in the diets of growing lambs had no effect on LWG, while influenced the final live weight (FLW), dry matter intake (DMI) and FCR. The highest FLW was seen when 100 g RW kg^{-1} DM was included in the diet (39.2 *vs*. 44.2 for 0 and 100 g RW kg^{-1} DM, respectively; L, $p = 0.006$). Dry matter intake in the RW-fed lambs was lower than in the control group (1,203 *vs*. 1,303 g day^{-1} in RW-fed and control-fed lambs, respectively; $p = 0.032$). Also, inclusion of RW in the diet improved the FCR ($p = 0.027$). The expenses of feed were € 0.23, 0.21, 0.20 and 0.18 kg^{-1} of DM of diet for R_0, R_1, R_2 and R_3, respectively. Addition of RW is estimated to decrease feed costs (€ kg^{-1} gain) by 30% for R_1 and 41% for R_3, respectively. Ammonia concentration was decreased quadratically when diets contained 200 or 300 g of RW kg^{-1} DM (Table 3). The highest concentration of ammonia was observed in R_1 (2.56 mmol

Table 2. Effect of different inclusion levels of raisin wastes (RW) on final live weight (FLW), live weight gain (LWG), dry matter intake (DMI) and feed conversion ratio (FCR)

Parameters	Experimental diets[1]						Contrasts[3]		
	R_0	R_1	R_2	R_3	SEM[2]	*p* value	Control *vs.* RW	L	Q
ILW[4] (kg)	30.1	33.1	33.9	30.1	2.02	0.128	0.214	—	—
FLW (kg)	39.2	44.2	43.9	41.1	1.27	0.015	0.009	0.006	0.482
LWG (g day^{-1})	150	185	166	182	20.1	0.682	0.365	0.846	0.617
DMI (g day^{-1})	1,303	1,220	1,231	1,159	29.8	0.063	0.032	0.348	0.647
FCR (g g^{-1})	8.7	6.6	7.4	6.4	0.30	0.034	0.027	0.092	0.125

[1] R_0, R_1, R_2 and R_3 = 0, 100, 200 and 300 g RW kg^{-1} DM of diet, respectively. [2] SEM = standard error of the mean. [3] L = linear; Q = quadratic. [4] ILW = initial live weight.

Table 3. Effect of different inclusion levels of raisin wastes (RW) on total volatile fatty acid (VFA) and ammonia-N concentrations and numbers of ciliated protozoa in the rumen of Mehraban growing lambs

Parameters	Experimental diets[1]					Contrasts[3]		
	R_0	R_1	R_2	R_3	SEM[2]	Control *vs.* RW	L	Q
VFA (mmol L^{-1})	92.71	72.1	102.58	101.04	4.8	0.235	0.651	0.141
Ammonia (mmol L^{-1})	2.22	2.56	1.37	1.67	0.3	0.741	0.214	0.042
Protozoa[4]								
Entodinium spp.	5.68	5.64	5.73	5.76	0.09	0.031	0.046	0.254
Epidinium spp.	4.65	4.76	0.00	4.32	0.10	0.015	0.025	0.001
Dasytricha spp.	3.74	3.70	3.95	4.00	0.08	0.025	0.044	0.074
Isotricha spp.	3.00	3.70	2.48	3.70	0.11	0.001	0.041	0.082
Diplodinium spp.	0.00	4.17	3.85	3.79	0.12	0.012	0.001	0.001
Ophryoscolex spp.	0.00	2.60	2.70	0.00	0.09	0.014	0.049	0.001
Total	5.73	5.73	5.74	5.79	0.11	0.002	0.003	0.752

[1] R_0, R_1, R_2 and R_3: 0, 100, 200 and 300 g raisin wastes (RW) kg^{-1} DM of diet, respectively. [2] SEM = standard error of the mean. [3] L = linear; Q = quadratic. [4] Because the protozoal population was not normally distributed among animals and treatments, the data were log-transformed.

L^{-1}), while the lowest value was recorded for R_2 (1.37 mmol L^{-1}).

Dietary inclusion of RW increased the concentration of *Entodinium spp.* and total number of rumen protozoa (Table 3). When the RW was included in the diet, *Epidinium ssp.* disappeared in the rumen fluid samples (L, $p = 0.025$; Q, $p = 0.001$) and low numbers of *Ophryoscolex spp.* were observed when RW was included in the diets at 100 and 200 g RW kg^{-1} DM.

Experiment II

The apparent total tract digestibility of CP and NDF decreased when RW was included in the diets (Table 4). The highest CP and NDF digestibility were observed for the control diet and the lowest digestibility values of CP and NDF were recorded for R_3 and R_2, respectively (Table 4). Addition of RW quadratically decreased apparent digestibility of CP ($p = 0.009$) and NDF

Table 4. Effect of different inclusion levels of raisin wastes (RW) on dry matter (DM), organic matter (OM), crude protein (CP) and, neutral detergent fiber (NDF) apparent digestibility and nitrogen retention in Mehraban growing lambs

	Experimental diets[1]				Contrasts[3]			
	R_0	R_1	R_2	R_3	SEM[2]	Control *vs.* RW	L	Q
Apparent digestibility (g k^{-1} DM)								
DM	769	760	735	727	710.2	0.142	0.652	0.746
OM	754	757	755	759	8.3	0.215	0.459	0.134
CP	879	847	792	781	50.7	0.044	0.118	0.009
NDF	631	620	572	588	49.4	0.049	0.132	0.041
Nitrogen balance (g day^{-1})								
Consumed N	32.5	31.2	30.9	23.2	2.21	0.037	0.664	0.452
Faecal N	4.5	5.1	6.7	5.3	0.41	0.018	0.741	0.048
Urinary N	2.7	2.4	2.4	1.9	0.32	0.036	0.039	0.254
Retained N	25.3	23.7	21.9	16.1	2.15	0.019	0.652	0.042
Faecal endogenous N	3.2	3.4	2.9	3.5	0.59	0.254	0.745	0.374
Efficiency of N retention[4] (g kg^{-1} N)	780	757	700	683	6.24	0.036	0.451	0.008

[1] R_0, R_1, R_2 and R_3 = 0, 100, 200 and 300 g raisin wastes per kg DM of diet, respectively. [2] SEM = standard error of the mean. [3] L = linear; Q = quadratic. [4] Calculated as N retained/N ingested.

($p = 0.041$). Apparent digestibility of CP ($p = 0.044$) and NDF ($p = 0.049$) was lower in the RW-fed lambs than in the R_0 lambs (Table 4).

Except for faecal endogenous nitrogen, the parameters of nitrogen balance trial significantly differed between the RW-fed lambs and the R_0 (Table 4). Inclusion of RW in the diet lowered the intake of N, UN, retained N and efficiency of retained N compared to the R_0 diet. Increasing levels of RW in the diet increased the FN excretion (Table 4). Inclusion of RW in the diet, quadratically decreased the intake of N from 32.5 g kg^{-1} DM in the R_1 lambs to 23.2 g kg^{-1} DM in the R_3-fed lambs ($p = 0.037$). UN decreased by 30% when the level of RW increased up to 300 g RW kg^{-1} DM in the diet (L, $p = 0.039$). In contrast, at inclusion level of 200 g RW kg^{-1} DM of diet the highest faecal N excretion was observed compared to the control group (Q, $p = 0.048$).

Discussion

The FLW in the R_1 and R_2 was higher than in the other groups, which partly could be due to the higher initial weight of animals in the R_1 and R_2 groups. Although the initial weights of R_0 and R_3 were statistically similar, the FLW in R_3 was numerically higher than in R_0. A part of RW was composed of rejected raisins which contains high concentration of soluble carbohydrates. Dietary inclusion of RW elevated the nitrogen free extract contents of diets, and hence increased their nutritive values. The improved FLW in the R_2 and R_3-fed lambs may also be attributed to the presence of tannins in RW. It has been reported that the presence of 4% tannin in the diet improves the performance of animals (Waghorn *et al*., 1990). However, Priolo *et al*. (2000) showed that 2.5% of condensed tannins from carob pulp had a deleterious effect on feed efficiency and nutrient digestibility of lambs. The measured TP in RW was lower than the value reported by Waghorn *et al.* (1990), but the bound tannins which are released slowly during fermentation of cell wall in the rumen may have exerted some beneficial effects (Makkar *et al*., 1997). Alipour & Rouzbehan (2007) reported that the amount of fiber bound tannins in grape pomace was higher than that of extractable tannins (2.7 *vs.* 1.5%, respectively). Also, smaller particle size of RW compared to alfalfa hay and barley grain may have enhanced the interaction between microorganisms and feed surface (Dehority, 2003). This process is necessary for cell wall digestion and results in higher nutrient availability for animals (Miron *et al*., 2001).

Reduction in DMI after addition of RW could be due to the presence of tannins which causes astringency in oral cavity and therefore decreases palatability (Reed, 1995). Similar to our results, Bahrami *et al.* (2010) found that using dried grape pomace in the diet of growing lambs decreased DMI. Hervas *et al*. (2003) reported that when ewes were intra-ruminally dosed with quebracho condensed tannins at 3 g kg^{-1} of body weight while fed alfalfa hay, they had a 95% reduction in DMI after 3 days of dosing. The lower DMI in the R_3-fed lambs compared to the other lambs may also explained by the lower dietary protein of R_3. Dabiri & Thonney (2004) reported a lower DMI in lambs receiving 13% protein compared to those received diets containing 15% protein. The CP content of diets is often related positively to DMI (Roffler *et al*., 1986). This is partly due to increased rumen degradable protein which affects feeds' digestibility (Oldham, 1984), and the mechanism presumably involves a reduction in distension as fiber and DM digestibility increase.

Dietary inclusion of RW decreased digestibility of NDF and CP, and the lowest values were observed for the R_3. Some factors such as soluble carbohydrates, amount and source of dietary protein influence fiber digestion in the rumen (Van Soest, 1994). Also, some genera of protozoa have a more important role in the carbohydrate digestion (Dehority, 2003). Takenaka *et al.* (2004) reported that *Entodinium* spp. have weak fibrolytic activities, while strong fibrolytic activities were observed for *Epidinium* spp. Michalowski *et al*. (2001) showed that *Epidinium ecaudatum* has strong fiblolytic activity for carboxymethylcellulose and xylan. When the RW was included in the diet at the level of 200 g kg^{-1} DM, *Epidinium* spp. completely disappeared, while concentration of *Entodinium* spp. and total number of protozoa were increased. Since the axenic culture of rumen protozoa are difficult to maintain, the interpretation of many results which are obtained in *in vivo* experiment are impossible. The responses of different protozoal genera to varying levels of RW were different, and the mechanisms by which the protozoa are affected not well understood. Both inhibitory and stimulatory effects of tannins on rumen protozoa have been reported (Patra & Saxena, 2009). In a review by Patra & Saxena (2009) it was pointed out that most researchers have found a decrease in the number of protozoa after feeding tannin containing diets. However, similar to our results Chiquette *et al.* (1989) reported that the total number of protozoa increased when the lambs fed increasing levels of birdsfoot trefoil (*Lotus coniculatus*).

Decreased protein digestibility in the RW-containing diets could be partly due to presence of tannins which increased faecal nitrogen excretion. Similarly, Carulla *et al*. (2005) found that addition of tannin to the diet of forage-fed sheep changed the excretory pattern of nitrogen from urine to faeces. This can be advantageous, because the UN is prone to ammonia emission during manure storage (Śliwiński *et al*., 2004). In this respect the advantages of tannins can be the reduction of readily volatile UN and/or continuing their protein binding activity during manure storage (Śliwiński *et al*., 2004).

Daily DMI was positively correlated ($p = 0.009$) with nutrient digestibilities ($p = 0.008$), consumed N ($p = 0.034$), faecal N ($p = 0.046$), retained N ($p = 0.037$) and efficiency of retained N ($p = 0.026$). Also, FCR had a strong and positive relation with digestibility of DM, OM, CP ($p < 0.01$) and NDF ($p = 0.021$). The lack of correlation between some measured parameters could be due to the presence of tannins in RW which led to changed rumen microbial population and diversity (Reed, 1995). This factor may also cause some alterations in the digestion and excretion of nutrients (*e.g.* nitrogen) which leads to difference between responses of animal receiving diets with or without tannins (Makkar, 2003a).

The results of this study showed that RW can be included in the diet of growing lambs at the level of 100 g kg^{-1} DM without adversely affecting the digestibility of nutrients. Feeding lambs with more than 200 g RW kg^{-1} DM reduced protein digestibility, nitrogen retention and absorption most likely due to the presence of tannins. 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 industry. Because of the low protein content of RW, this by-product must be fed with adequate protein supplements to meet the protein requirement of animals.

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