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Effects of Replacing Alfalfa Hay and Wheat Straw by Pistachio by-Product Silage and Date Waste on the Performance and Blood Parameters of Fattening Lambs

Research Article

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ABSTRACT

In this trial effects of feeding pistachio by-product silage with date waste (PBSD) on dry matter intake (DMI), live weight gain and blood parameters of lambs were studied. Ensiling of pistachio by-products (PBP) with date waste increased dry matter (DM) and metabolizable energy (ME), but decreased the percentages of crude protein (CP), total tannins and phenolic compounds ($P<0.05$). Treatment diets were: 1) control (without PBSD); 2) diet containing 7% PBSD; 3) diet containing 14% PBSD and 4) diet containing 21% PBSD (DM basis). Forty male lambs were allocated to four treatments in a completely randomized design. All groups were fed a control diet for 21 days prior to the start of the 90-day experiment. Thereafter, one group (control) continued to receive the same diet while other groups received the diets containing PBSD. At the end of experiment, all lambs were slaughtered on the same day. The lambs fed diets containing 14 or 21% PBSD had more DMI than those fed with the control diet ($P<0.05$). Feeding 21% PBSD significantly ($P<0.05$) increased final weight, live daily gain, the weight of the warm or cold carcass and lean meat. The lambs fed 14 and 21% PBSD had lower TG than lambs fed the control diet ($P<0.05$). Feeding 21% PBSD significantly ($P<0.05$) increased the blood total protein and albumin concentration when compared with the control group. The concentrations of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) enzymes in blood were not affected. The results showed that feeding 21% PBSD to fattening lambs increased their performance, and it had no adverse effects on liver function.

KEY WORDS blood parameters, date waste, fattening lamb, pistachio by-product silage.

INTRODUCTION

Climate change is now widely accepted as a real, pressing and truly global problem. The changes of climatic conditions and shortage of water have increased the cost of animal feeds in many countries. Using agricultural by-products, which are available after the processing of agricultural products, is often a useful way to overcome this problem (Bagheripour *et al.* 2008). Iran is one of the world's main pistachio producers. There is about 298939 ha of pistachio garden in Iran and annual dry pistachio production is about 307036 tonnes (Salehi *et al.* 2012). Every year up to 400000 tonnes of pistachio by-product (PBP) are produced in Iran, which deteriorate in open fields due to high moisture content (Norouzian and Ghiasi, 2012). Using PBP as an alternative animal feed will not only meet the feed shortage but also reduce the risk of environmental pollution (Gholizadeh *et al.* 2010). PBP which is composed of soft external hulls, twigs, leaves and some kernel and bony shells, remains from the de-hulling process of the crop. It is about 1.25-2 kg/kg dry pistachio and (Forough Ameri and Shakeri, 2008). The CP content of PBP varies from 92 to 120 g/kg on a dry matter (DM) basis, NDFom from 300 to 360 g/kg DM and ADFom from 210 to 280 g/kg DM (Forough Ameri and Fazaeli, 2005). The ME content of PBP may vary from 7.1 to 7.5 MJ/kg DM and its DM degradability was 463 g/kg (Forough Ameri and Fazaeli, 2005).

Date palm (*Phoenix dactylifera*) is one of the oldest fruit crops grown in the arid regions of the Arabian Peninsula, north Africa and the Middle east (Chao and Krueger, 2007). Date waste contains carbohydrates; thus, it may be possible to use it as an energy source for ruminants. It can provide 2.67 Mcal ME/kg DM (AlKhateeb and Ali-Dinar, 2002), thus, it may be possible to use it as an additive during silage making.

Ensiling PBP is one of the best methods for long-term preservation of this by-product as its high moisture content makes preservation difficult. To avoid spoilage, to reduct total tannins and phenolic compounds and to increase nutritional value, PBP can be ensiled with date waste and used in feeding ruminants. The objective of this study was to investigate the effect of replacing alfalfa hay and wheat straw by ensiled pistachio by-products with date waste on performance and blood parameters in fattening lambs.

MATERIALS AND METHODS

Product silage with date waste (PBSD)

During pistachio harvest season, 2000 kg of fresh PBP and 400 kg of date waste (90% DM, 97.6% OM, 7% CP, 0.35% EE, 23.3% NDF, 17.2% ADF and 2.9 Mcal/kg ME) were ensiled. After two months, samples were collected using a specific probe from different parts of the silo. Samples were freeze-dried and then ground using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA, USA) to pass through 1 mm mesh. According to AOAC (2000), ground composited samples were analyzed for DM and CP. The neutral detergent fiber of organic matter (NDFom) and acid detergent fiber of organic matter (ADFom; Van Soest *et al.* 1991) were determined. For a tannin assay, samples were dried at 40 ˚C to constant weight to minimize changes in tannin content and activity and dried samples were ground through 0.5 mm mesh before analysis (Makkar, 2003). Phenolic compounds were extracted using 200 mg of each dried sample. For extraction the sample was made up to 10 mL with aqueous acetone water (700:300, v/v), then left at 4 ˚C overnight. Extracts were centrifuged at 3000 g at 4 ˚C for 15 min, then the supernatant analysed. The concentration of total phenolic compounds (TP) was determined using the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) and the regression equation of tannic acid (Merck GmbH, Darmstadt, Germany) standard. Total tannins (TT) were estimated indirectly after being absorbed to insoluble polyvinyl polypyrrolidone (PVPP). The concentration of TT was calculated by subtracting the TP remaining after the PVPP treatment (Makkar, 2003).

Silage pH was measured using an electronic pH meter. Fleig point was calculated using the following formula (Denek and Can, 2006):

Fleig point= 220 + (2×% dry matter-15) - 40 × pH

Animals and diets

40 six month old Kermani male lambs (average live weight 21±1.4 kg) were used to this trial. Lambs were allocated to four treatments of 10 / treatment in a completely randomized design. Housing was in individual pens with straw bedding in a sheltered, cement-floored, open-sided, well-ventilated barn. Fresh, clean water was available at all times. All lambs ate a control diet for 21 days before the 90-day experiment. Thereafter, one group (control) continued to receive the same diet while other groups received diets containing PBSD. The lambs were fed TMR diets *ad-libitum* twice daily (08:00 and 17:00 h), then the amounts of feed offered were recorded and adjusted according to feed refusals daily. The animals were maintained according to guidelines set by the Iranian Council of Animal Care (1995). All lambs were sheared and treated with albendazole (Roacel) for internal parasites and vaccinated against enterotoxemia (Razi Vaccine and Serum Research Institute). Clinical examination during the study found that all lambs were in good conditions with no case of severe clinical disease.

Diets were as follow: 1) control (without PBSD); 2) diet containing 7% PBSD; 3) diet containing 14% PBSD and 4) diet containing 21% PBSD (DM basis). All diets contained 40% of forage and 60% of concentrate. Mineral and vitamin additions were the same in all diets. Ingredients and nutrient composition of the diets is shown in Table 1. Samples of the diets were ground (1 mm screen) and analyzed for DM, CP and EE by the AOAC (2000) method. The neutral detergent fiber of organic matter (NDFom) and acid detergent fiber of organic matter (ADFom; Van Soest *et al.* 1991) were determined.

Individual intakes were calculated using daily feed offered and feed refused averaged over the interval of the performance phase. Live weights, determined by removing feed at 05:00 and weighing lambs at 8:00, were obtained on the performance phase weekly. Individual average daily gain was calculated as the difference between initial and final weights over the interval of the performance phase (90 d). On the last day of the trial (Day 90), two hours before feeding, blood samples were collected in 10 mL vacutainers. The vacutainers were transported to the laboratory immediately. Serum was separated after centrifugation at 750 g for 15 min and the serum samples stored at -20 ˚C until analysis.

Table 1 The ingredients and chemical composition of treatment diets (DM basis)

Ingredients	PBSD level (%)			
	0	7	14	21
Alfalfa hay, chopped	30	25	19	14
Wheat straw, chopped	10	8	7	5
Pistachio by-products silage with wasted date	0	7	14	21
Barley grain, ground	27	28	27	27
Corn grain, ground	13.5	13	10.5	9
Soybean meal	7.5	8	9.5	11
Wheat bran	9	8	10	10
Dicalcium phosphate	1	1	1	1
Vitamin A, D and E premix[a]	0.7	0.7	0.7	0.7
Trace-mineralized salt[b]	0.7	0.7	0.7	0.7
Sodium bicarbonate	0.3	0.3	0.3	0.3
Limestone	0.3	0.3	0.3	0.3
Chemical composition				
Dry matter (DM) (%)	87	85	81	77
Metabolizable energy (Mcal/kg DM)	2.51	2.27	2.57	2.58
Crude protein (%)	14.03	13.98	13.87	13.76
Ether extract (%)	2.60	2.83	3.07	3.20
NDFom (%)	35.72	33.09	32.13	30.95
ADFom (%)	21.45	19.48	18.45	17.28
Total tannins	0.27	0.59	0.93	1.25
Total phenolic compounds	0.61	1.26	1.88	2.55

[a] Contains (per kg): vitamin A: 5000000 IU; vitamin D: 5000000 IU and vitamin E: 500000 IU.
[b] Composition: NaCl: 75.15%; Dynamad: 20.5%; Mn: 3.046%; Cu-sulphate: 1.025%; Zn-sulphate: 0.253%; EDDI-80: 0.015% and Na-selenide: 0.011%.
PBSD: product silage with date waste; NDFom: neutral detergent fiber of organic matter and ADFom: acid detergent fiber of organic matter.

Serum biochemical analysis was done for measuring glucose by the glucose oxidase method (Lott and Turner, 1975), creatinine by the modified Jaffe method, blood urea nitrogen (BUN) by the diacetyl monoxime method (Burtis and Ashwood, 1994), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities by a method modified from that of Reitman and Frankel (Thrall *et al.* 2004), cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (ZiestChem Diagnostics Tehran, Iran) and triglyceride by the enzymatic procedure (ZiestChem Diagnostics Tehran, Iran) of McGowan *et al.* (1983). Lipoproteins were isolated using a combination of precipitation and ultracentrifugation. High density lipoprotein (HDL)-cholesterol was measured using the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins, which were sedimented by centrifugation (10000 g for 5 min). The residual cholesterol was then measured by the enzymatic method. Low density lipoprotein (LDL)-cholesterol was calculated as the difference between the cholesterol measured in the precipitate and in the HDL fraction. VLDL-cholesterol was estimated as 1/5 the concentration of triglycerides (Rucker *et al.* 2008). Serum total protein and albumin were measured by the Biuret method (Latimer *et al.* 2003). The results of total protein and albumin levels were used to calculate globulin.

Carcass analyses

At the end of the experiment, all lambs were slaughtered on the same day in the Kerman city slaughterhouse. After lambs were bled, they were pelted and the head severed at the atlas joint. All carcasses were hung by the Achilles tendon after slaughter. Warm and cold (i.e. after 24 h chilling at 4 ˚C) carcass weights without the head were recorded. The dressing percentage was calculated as weight of carcass/live weight at slaughter × 100. Cooler shrink refers to the loss of carcass weight between 0 and 24 h. The carcasses were halved longitudinally.

Statistical analyses

Data from chemical analysis of PBP and PBSD were subjected to analysis of variance as a completely randomized design. The mixed procedure (SAS, 2005) was used to determine statistical differences between diets in growth performance data. Initial live weight was used as a covariate for final body weight. The Tukey test was used to compare means, considering effects to be significant at ($P \leq 0.05$).

RESULTS AND DISCUSSION

The chemical compositions of PBSD and PBP diets are shown in Table 2. Ensiling of PBP with date waste increased the DM percentage and metabolizable energy concentration, but decreased the percentages of crude protein, total tannins and phenolic compounds ($P<0.05$). Ensiling did not affet the concentrations of NDFom and ADFom of PBP. DMI in lambs fed diets containing 14 and 21% PBSD were higher than that of the lambs fed the control diet (Table 3). There were no significant differences in initial body weights, but the final body weight of lambs fed the diet containing 21% PBSD was higher than other groups. Feeding 14 and 21% of PBSD increased live weight gain.

The weight of lean meat were higher in lambs fed with 14% and 21% of PBSD than in the control group (Table 3). Adding PBSD in diets 2, 3 and 4 compared with control diet had no significant effect on bone weights.

Feed conversion, warm and cold dressing percentages, and cooler shrink were not affected by feeding PBSD. Both warm and cold carcass weights were affected by the treatment diets (Table 3). Feeding 21% PBSD increased warm and cold carcass weights compared with the control group.

Table 2 The chemical composition, fleig point and pH of the pistachio by-products silage with wasted date (PBSD) and pistachio by-product (PBP) (n=5)

Item	By-products		SEM	P-value
	PBP	PBSD		
Dry matter (DM) (%)	27.3	33.2	0.86	0.024
Crude protein (CP) (% of DM)	10.26	8.3	0.89	0.028
NDFom (% of dry matter)	34.34	33.45	0.09	0.12
ADFom (% of dry matter)	24.37	22.65	0.18	0.19
Total tannins (% of dry matter)	7.42	4.82	0.02	0.008
Phenolic compounds (% of DM)	12.48	9.65	0.05	0.011
Fleig point	-	107.4	-	-
pH	-	4.10	-	-
Metabolizable energy (Mcal/kg DM)[a]	2.03	2.4	0.03	0.025

SEM: standard error of the means.

[a] Metabolizable energy (ME) (Mcal/ kg)= (0.027+0.0427 DDM) + 0.821 (Gonzalez and Everitt, 1982).

PBP: pistachio by-products; PBSD: product silage with date waste; NDFom: neutral detergent fiber of organic matter and ADFom: acid detergent fiber of organic matter.

The effect of feeding PBSD on blood parameters and liver enzymes of the lambs is shown in Table 4. The blood TG concentration of lambs fed diets containing 14 and 21% of PBSD were significantly lower than in lambs fed the control diet.

The blood total protein and albumin concentration were affected by the treatment diets. Feeding 21% of PBSD significantly increased blood total protein and albumin concentration when compared to the control group. No significant differences were observed for liver enzymes of serum glutamate-pyruvate transaminase (SGPT) SGPT and serum glutamate-oxaloacetate transaminase (SGOT) between lambs fed the treatment diets.

In the present study, adding date waste to PBP when ensiling increased silage DM, because the date waste had higher DM than PBP. Salehi *et al.* (2012) reported that ensiling of PBP increased the DM of silage. In another study, Islam *et al.* (2001) found that adding molasses to rye silage increased DM percentage. In contrast, Mokhtarpour (2009) reported that ensiling of PBP had no effect on DM and CP. Ensiling of PBP with date waste decreased CP, total tannins and phenolic compounds.

The concentrations of CP in date waste were lower than PBP, then adding it to PBP when ensiling decreased the CP content of PBSD.

Ensiling decreased intotal tannins and phenolic compounds concentrations in PBSD. Martens *et al.* (2014) reported that ensiling commonly reduced anti-nutritional compounds such as tannins by 49-84%. Vahmani *et al.* (2006) found that ensiling PBP could reduce the total tannins and phenolic compounds in PBP compared with sun-dried PBP. Thus, ensiling is a viable option to enhance nutrient utilization of PBP for ruminants.

Fleig point is a useful tool to evaluate silage quality. In this study, according to the equation of Denek and Can (2006), the fleig point of PBDS was estimated as 107.4, indicating very good quality. Generally, suitable silages pH is 3.5-4.5, while the pH of PBDS was 4.1 as determined.

Diets with different levels of PBSD affected DMI. Lambs fed 21% and 14% of PBSD consumed more DM than those fed the control diet. This is probably because: 1) the percentages of NDF and ADF of treatment diets decreased with increasing PBSD. The NDF concentration has a negative correlation with voluntary feed intake (Mertens, 2009). 2) Adding PBSD to diets decreased diets' DM content. PBP in animals' diets has been shown to have no effect on DMI (Ghasemi *et al.* 2012; Valizadeh *et al.* 2010; Rezaei *et al.* 2012) or to decrease it (Shakeri *et al.* 2004). Such discrepancies may be related to experimental conditions such as animal, concentration of fat, fiber, energy, dietary CP degradability and forage type may control DMI.

In the present study, feeding 21% PBSD increased daily live weight gain and final weights. On the other hand, the higher body weight of lambs fed 21% PBSD than other groups might be due to higher DM intake. In contrast, Norouzian and Ghiasi (2012) found that feeding dry PBP had no effect on carcass performance and daily gain in Balouchi lambs. According to Shakeri *et al.* (2012), the inclusion of PBP silage up to 18% of Holstein male calves' diets had no adverse effects on DMI, growth performance or blood metabolites after a long-term feeding program. Valizadeh *et al.* (2010) found that the addition of PBP up to 30% in fattening lambs' diets had no effects on daily weight gain and live weight gain. Rezaei *et al.* (2012) reported that feeding a diet containing 30% of dry PBP to fattening camels reduced daily weight gain and final weights.

Feeding diets with different levels of PBSD had no effect on feed conversion, because along with an increase in DMI, daily weight gain increased. Feed conversation ratio, which depends on diet, age, weight and breed, is 5-7 in Iranian meat breeds (Foroughi, 1996). Shakeri *et al.* (2004) reported similar feed conversion ratios when adding PBP to the diet of Kermani fattening lambs. In another study Valizadeh *et al.* (2010) found that up to 30% PBP in the diet of Balouchi lambs had no significant effects on feed conversion ratio.

Rezaei *et al.* (2012) found that feed conversation ratio in fattening camels was not affected by diets containing dry PBP up to 30%. In contrast, Shakeri (2016) reported that feeding of sun-dried PBP up to 30% in Kermanian male lambs increased feed conversion ratio linearly.

The weights of warm and cold carcasses and lean meat were higher for the lambs fed a diet containing 21% PBSD than in those fed the control diet. These observation can be attributed to a higher final weight after eating the diet containing 21% PBSD.

found that the addition of PBP up to 18% Holstein male calves' diet had no effects on blood TG.

Table 3 Performance and dry matter intake of lambs fed treatment diets

Item	PBSD level (%)				SEM	P-value
	0	7	14	21		
Dry matter intake (kg/day)	1.08[b]	1.14[ab]	1.17[a]	1.22[a]	0.03	0.003
Average initial weight (kg)	21.1	21.2	21.2	21.4	1.42	0.951
Average final weight (kg)	35.8[b]	36[b]	36.3[b]	37.2[a]	0.23	0.002
Average live daily gain (g)	162.3[c]	163.4[c]	169.2[b]	176.1[a]	0.91	0.001
Average live weight gain (kg)	14.6[c]	14.7[c]	15.14[b]	15.8[a]	0.12	0.016
Warm carcass weight (kg)	16.89[b]	17.3[b]	17.72[ab]	17.97[a]	0.32	0.006
Cold carcass weight (kg)	16.2[b]	16.93[ab]	16.9[ab]	17.29[a]	0.25	0.023
Warm dressing percentage	47.07	47.89	48.46	48.24	0.90	0.459
Cold dressing percentage	45.34	46.85	46.20	46.52	0.60	0.562
Cooler shrink (% of warm carcass)	3.66	3.18	4.23	3.47	0.80	0.497
Lean meat (kg)	11.21[b]	11.9[ab]	12.14[a]	12.07[a]	0.32	0.012
Bone (kg)	3.16	3.09	3.25	3.2	0.10	0.750
Average feed conversion (kg dry matter/kg gain)[a]	7.16	7.19	7.38	6.61	0.70	0.063

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.
PBSD: product silage with date waste.
[a] Feed conversion was calculated as total dry matter intake (DMI) divided by total weight gain.

Table 4 The blood parameters of lambs fed treatment diets

Item	PBSD level (%)				SEM	P-value
	0	7	14	21		
Cholesterol (mg/dL)	65.6	59.6	61.2	63.8	3.04	0.466
HDL (mg/dL)	41.2	42.6	38.6	38.4	2.7	0.623
LDL (mg/dL)	47.34	48.12	46.71	47.89	1.34	0.463
TG (mg/dL)	60[a]	51.4[ab]	38.8[b]	34.4[b]	4.53	0.459
Glucose (mg/dL)	85.4	86.2	80.4	85.6	2.25	0.833
Blood urea (mg/dL)	46.84	44.85	46.08	47.23	1.66	0.451
Creatinine (mg/dL)	2.22	2.18	2.22	2.26	0.049	0.916
Total protein (g/dL)	6.08[b]	6.22[ab]	6.36[ab]	6.62[a]	0.14	0.002
Albumin (g/dL)	3.12[b]	3.36[ab]	3.42[ab]	3.70[a]	0.18	0.001
Globulin (g/dL)	2.92	2.85	2.96	2.91	0.09	0.341
SGPT (IU/L)	28.26	26.19	29.25	27.48	1.17	0.546
SGOT (IU/L)	113.63	111.30	110.54	109.92	1.95	0.430

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.
PBSD: product silage with date waste; HDL: high density lipoprotein; LDL: low density lipoprotein TG: triglycerides; SGPT: serum glutamic pyruvic transaminase and SGOT: serum glutamic oxaloacetic transaminase.

In contrast, Norouzian and Ghiasi (2012) and Valizadeh *et al.* (2010) reported that feeding of PBP up to 30% in fattening lambs diet had no effects on warm and cold carcass weights. Diets containing PBSD did not influence the cooler shrink of fattening lambs, but for lambs fed PBSD, it was similar to the required surface drying of 2-4% carcass weight loss, which is inhibitory to bacterial growth. More-O'ferral *et al.* (1989) reported highly significant differences in cooler shrink between breeds and suggested that the higher cooler shrink indicates poorer water-holding capacity of the muscle, which could have commercial significance for the packaging trade.

In the present study, lambs fed 14 and 21% PBSD had lower triglycerides (TG) than lambs fed the control diet. Gholizadeh *et al.* (2010) reported that PBP in lambs' diet decreased the blood TG concentration. Shakeri *et al.* (2012)

The blood total protein and albumin concentrations were higher in lambs fed the diet containing 21% PBSD than in those fed control diet. These observations can be attributed to higher tannin concentration in diet containing 21% PBSD compare to control diet, which is decreasing the rate of protein degradation in the rumen. In contrast, Shakeri *et al.* (2012) reported that feeding male calves 12% and 18% PBP decreased the blood albumin concentration.

The concentrations of liver enzymes in the blood of fattening lambs as indicators of liver function were not affected by feeding treatment diets. This is supported by Alemu *et al.* (1977), who found that liver damage in ruminants often resulted in high serum SGOT and only slightly raised SGPT. Shakeri (2016) reported that adding sun-dried PBP up to 30% in Kermanian male lambs' diet had no effects on the plasma activities of circulating enzymes including alanine aminotransferase and aspartate aminotransferase.

CONCLUSION

The results of this study indicate that ensiling PBP with date waste reduced total tannins and phenolic compounds. Furthermore, using 21% of PBSD in replacement for alfalfa hay and wheat straw, in fattening lambs' diet, improved the dry matter intake and animals' performance.

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