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The effects of feeding alfalfa pulp ensiled with wasted date (*Phoenix dactylifera L.*) on digestibility, microbial protein synthesis and ruminal fermentation characteristics in sheep

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Abstract The aim of this study was to evaluate the effect of feeding alfalfa pulp ensiled with waste date (*Phoenix dactylifera L.*) on digestibility, microbial protein synthesis and ruminal fermentation characteristics in Kermani sheep. Alfalfa (*Medicago sativa L.*) pulps were ensiled with waste date (15% in dry matter) in buckets. After 45 days, chemical composition and pH of the silage were evaluated using four Kermani rams in a change-over design with four 21-day periods comprising of 16 days for adaptation and 5 days for sample collection. Treatments containing 4 diets: 1) control diet (without silage); 2) diet containing 10% silage; 3) diet containing 20% silage and 4) diet containing 30% silage. The results of this study showed that adding 15% waste date to alfalfa pulp during ensiling, improved silage quality and DM. The Flieg point and pH of silage were 94.26 and 3.8, respectively, with a total score quality evaluation of 19 that seemed to be a very good score. Nutrient digestibility, nitrogen (N) retention, blood parameters, urinary purine derivatives and microbial protein synthesis were not affected by treatments. The total population of Entodinium and total protozoa species were increased linearly with the increase in the level of alfalfa pulp ensiled with waste date in the diets. In conclusion, ensiling of alfalfa pulp with 15% waste date increased DM and silage quality and its feeding to sheep did not have negative effects on feed intake and nutrient digestibility. Due to the relatively low costs of alfalfa pulp and waste date, their inclusion in sheep diets can reduce the cost of feed and environmental pollution.

Keywords: silage, Flieg point, digestibility, purine, protozoa

Introduction

A key strategy for continuance of livestock development is the efficient use of available feed resources, particularly for those which are not in competition with human food like the agricultural by-products (Rajabi et al., 2016). Alfalfa pulp is a by-product of alfalfa extraction that has not been used by humans, and its accumulation may cause environmental pollution. Alfalfa (*Medicago sativa L.*) is a well-adapted high quality grass with high nutritional value and palatability (Jian et al., 2015). The protein in alfalfa silage is particularly problematic, being subject to extensive degradation to non-protein nitrogen (NPN) in the silo (Broderick and Muck, 2009). The NPN contents in alfalfa silage range from 43% (Nagel and Broderick, 1992) to as much as

87% (Muck, 1988) of total protein equivalent. Extensive protein degradation in the silo may lead to both reduced N efficiency in ruminants (Coblentz and Grabber, 2013; Repetto et al., 2005), raising environmental concerns regarding excess urinary N excretion (Hartinger et al., 2019).

However, due to its high crude protein (CP), low carbohydrate, and higher buffering capacity, it hardly meets the needs of lactic acid fermentation in the silage process, and this makes it difficult for the single alfalfa to succeed in silage (Yang et al., 2004; Coblentz and Muck, 2012). Recent researches showed that the use of mixed silage could help complement nutrients between silages, such as alfalfa and corn (Wang et al., 2011), alfalfa and waste date (Rajabi et al., 2016), alfalfa and sorghum-Sudan grass (Xue et al., 2013), alfalfa and Italian Ryegrass (Wen et al., 2011), alfalfa and bulrush (Zeng et al., 2011), which had better fermentation effect and more balanced nutrients than alfalfa silage alone. Due to high humidity, alfalfa pulp is vulnerable to deterioration; therefore, by ensiling with additives, it can be stored for a longer period of time (Chaudhry and Naseer, 2006).

The date palm (*Phoenix dactylifera L.*) is rich in carbohydrates (total sugars, 44–88%, fat (0.2–0.5%), CP (2.3–5.6%), dietary fiber (6.4–11.5%), minerals (0.1 to 916 mg/100 g date) and vitamins such as C, B1, B2, A, riboflavin and niacin (Al-Hooti et al., 1997). The major regions of date production are in the Middle East and North Africa. Iran is the world's second largest producer of dates. In 2013, it produced 1.08 million tones that make 14% of world's date production (FAO, 2016). Waste date pulp, low grade rejected date fruits and the date seeds (pits, stones) are the three major by-products of the date fruit processing plants (Sidhu, 2012). Approximately, 20–30% of total harvested date fruits are wasted date which are not suitable for human consumption and may be used as an ingredient in the diets of small ruminants because of inadequate texture (too soft or too hard), or simply due to their low quality (MAJ, 2016). Waste date can be used as a carbohydrate source during alfalfa ensiling. It seems that waste date can be used in ensiling alfalfa pulp as a soluble carbohydrate source for rapid reduction of the pH, and microbial access; therefore, this study aimed to evaluate the effect of feeding the ensiled alfalfa pulp with waste date on ruminal fermentation characteristics and microbial protein synthesis in Kermani sheep.

Materials and methods

Silage production and quality

Alfalfa pulp was obtained from Parsa Gol's factory in Baft, Kerman, Iran (latitude 28°49' N, longitude 56°19' E) and ensiled with waste date at 15% DM level in buckets (45-liter capacity) for 45 days, after which the chemical composition and pH of the silage were determined.

The DM, organic matter (OM), ash, crude fat and crude protein (CP) contents of the silage were determined according to the AOAC (1990) method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991) method (Fiber Tec system 2010; Foss Company, Hillerød, Denmark). Table 1 shows the chemical composition of alfalfa pulp and waste date.

The pH of each sample was determined in triplicate using approximately 25 g wet ensilage added to 100 mL of distilled water. After hydration for 10 min, pH was determined using a digital pH meter (Elmetron-CP 103). Physical features, such as color, smell, and structure and quality classification of the silage were determined by total points. As a result of physical analysis, silage with a score of 16-20 points, 10-15 points, 5- 9 points, and 0-4 scores were classified into very good, good, medium and bad quality, respectively (Kilic, 1986). The Flieg points of the silage were calculated by the following equation reported (Kilic, 1984).

$$\text{Flieg Point} = 220 + (2 \times \%\ \text{DM} - 15) - 40 \times \text{pH} \qquad (1)$$

where Fleig points denote that values between 85 and 100 signify very good quality; 60 and 80, good quality; 55 and 60, moderate quality; 25 and 40, satisfying quality; <20, worthless.

Animals and diets

The experimental treatments consisted of 4 diets: 1) control diet (without silage), 2) diet containing 10% silage, 3) diet containing 20% silage and 4) diet containing 30% silage (Table 2). All diets contained 40% forage and 60% concentrate.

Four Kermani rams (36 ± 2 kg live weight) were used in a change-over design with four 21-day periods

Table 1. Chemical composition (%) of alfalfa pulp and waste date without kernel

Item	DM	OM	CP	Ash	EE	NDF	ADF
Alfalfa pulp	22.23	87.50	15.24	12.50	6.00	57.07	46.88
Waste date	95.92	93.00	6.28	7.00	5.75	24.13	15.50

Table 2. Ingredients and chemical composition of the experimental diets (DM basis)

Ingredient (%)	Experimental diets[1]			
	0	10	20	30
Alfalfa hay	45	35	25	15
Wheat straw	15	15	15	15
Alfalfa pulp silage	0	10	20	30
Barley grain, ground	25	25	25	25
Corn grain, ground	6	5.5	5.25	5
Soybean meal	2	2.5	2.75	3
Wheat bran	5	5	5	5
Vitamins and minerals premix [2]	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5
Chemical composition				
Metabolizable energy (Mcal/kg DM)	2.41	2.43	2.44	2.46
Crude protein (%)	12.24	12.25	12.19	12.13
Dry matter (%)	88.55	82.26	75.97	70.39
Organic matter (%)	90.80	90.75	90.71	90.69
Ether extract (%)	2.01	2.36	2.71	3.06
Neutral detergent fiber (%)	42.66	41.77	40.88	39.99
Acid detergent fiber (%)	29.06	28.55	28.05	27.53
Non-fiber carbohydrates (%)	32.73	34.08	35.80	38.13

[1]0: Control diet (without silage), 10: diet containing 10% silage, 20: diet containing 20% silage and 30: diet containing 30% silage.
[2]Contained (per kg): 500,000 IU of vitamin A; 100,000 IU of vitamin D and 100 IU of vitamin E, 3000 mg Fe, 300 mg Cu, 300 mg Mn, 2000 mg Ca, 3000 mg Zn, 90000 mg P, 100 mg Co, 50000 mg Na, 100 mg I, 19000 mg Mg and 0.1 mg Se.

consisting of 16 days for adaptation to the diet and 5 days for sample collection. The sheep were housed individually in metabolic cages for fecal and urinary collection. Diets were offered as a total mixed ration (TMR) at 8:00 and 16:00 h. Water was freely available. The management and care of animals were in accordance with the guidelines and recommendations of the Iranian Council of Animal Care (1995).

Blood, fecal, urinary and ruminal fluid sampling

On the last day of each experimental period, blood was collected four hours after morning feeding from the jugular vein in 10 mL syringes and immediately transferred into 10-mL sodium heparinized, evacuated glass tubes and placed on crushed ice. Blood was centrifuged at 3,000 g for 10 min at 4°C, and plasma harvested and frozen at −20 °C for later analysis. Glucose, total protein, triglycerides, cholesterol and blood urea nitrogen (BUN), were measured using enzymatic procedures and commercial kits according to the manufacturer's instructions (Pars Azmon Co., Tehran, Iran). Total feces were collected in plastic bags every five days during the experimental period. After collection, the feces were weighed and dried in an oven at 55 ºC for 72 h, for DM, OM, CP and NDF analysis. Daily urine production was collected for 5 d and a 100 mL sample was mixed with 10% (V/V) H_2SO_4 to prevent bacterial degradation of allantoin and volatile N losses. Due to the variability in the urine volume produced by rams, the volume of H_2SO_4 was adjusted to ensure that the pH was maintained below 3.0 (Gomes et al., 2014). To determine the urinary purine derivatives, uric acid, urea N and creatinine excretion, a 50-L sub-sample of the diluted urine was stored in a plastic bottle at -20 ºC. At the completion of each period, ruminal fluid samples were aspirated by a stomach tube at 0, 2, 4 and 6 h after feeding. The ruminal pH was determined immediately after rumen fluid was filtered through four layers of cheesecloth using a digital pH meter (Elmetron-CP 103). A 10-mL sample was mixed with 0.2 mL of sulfuric acid 50% (Merck, Germany) for NH3-N analysis, and chilled in an ice bath to stop fermentation. The samples were stored at -20 ºC until assay. Ciliated protozoa were counted by a Neubauer improved bright-line counting cell (0.1 mm depth, Hausser Scientific, Horshman, PA, USA) in frozen-thawed rumen fluid samples. Collected samples were preserved with methylgreen-formalin-saline (MFS) solution (Ogimoto and Imai, 1981). Each sample was counted in duplicate. The numbers of different genera in population were recorded and grouped as Entodinium sp., Holotrichs (Isotricha and Dasytricha sp.) and cellulolytic protozoa (Polyplastron, Diplodinium and Enoploplastron sp.) as described by Dayani et al. (2007).

Statistical analysis

Chemical composition data were subjected to analysis of variance using the GLM procedure of SAS (2005) as a completely randomized design as $Y_{ij}= \mu + T_i + e_{ij}$ where, Y_{ij} is the dependent variable, μ is the general mean, T_i the ith effect of the treatments and e_{ij} is the standard error. Data on digestibility, blood parameters, etc. were analyzed using the following model:

$$Y_{ijk} = \mu + T_i + P_j + C_k + e_{ijk} \quad (2)$$

Repeated measure data (ruminal pH and ammonia-N) were analyzed using the following model:

$$Y_{ijkm} = \mu + T_i + P_j + C_k + Z_m + ZT_{mi} + e_{ijkm} \quad (3)$$

where, Y_{ijk} = dependent variable, μ = population mean, T_i = treatment effect, P_j = random effect of period, C_k = random effect of animal, Z_m = time effect, ZT_{mi} = interaction effect of treatment and time and e_{ijk} = random error.

Polynomial contrasts were used to test for linear, quadratic and cubic effects of level of alfalfa pulp ensiled with 15% waste date. Mean separation was performed using the least significant difference (P<0.05).

Results

Table 3 shows the chemical composition of alfalfa pulp and alfalfa pulp ensiled with 15% waste date. Alfalfa pulp ensiled with waste date contained higher (P<0.01) DM than alfalfa pulp (28.13% vs. 22.23%). No statistically significant difference was found in OM, CP, ash, EE, NDF, and ADF contents between alfalfa pulp and alfalfa pulp ensiled with 15% waste date.

The Flieg point and pH of the ensiled materials were 94.26 and 3.8, respectively, indicative of high quality silage. The score for color, smell, texture and total score were 2.0, 13.0, 4.0 and 19.0, respectively, and a total score of 19 was a very good score.

As shown in Table 4, there was no statistically significant difference between the treatments with respect to DM and N intake, fecal and urine N and N retained.

No statistically significant difference was found between the experimental treatments with respect to digestibility of DM, OM, CP, and NDF (Table 5).

Table 3. Chemical composition (%) of alfalfa pulp and alfalfa pulp ensiled with 15% waste date

Items	Alfalfa pulp	Alfalfa pulp ensiled with 15% waste date	SEM[1]	*P* value
Dry matter	22.23	28.13	0.03	0.005
Organic matter	87.50	89.25	0.17	0.09
Crude protein	15.24	14.16	0.38	0.29
Ash	12.50	10.75	0.17	0.09
Ether extract	6.00	5.62	0.08	0.20
Neutral detergent fiber	57.07	45.91	3.04	0.23
Acid detergent fiber	46.88	37.15	1.30	0.11

[1]SEM= Standard error of the mean.

Table 4. Dry matter intake, nitrogen intake, nitrogen excretion (fecal and urinary) and nitrogen retention in sheep fed treatment diets

Items	Diets[1]				SEM[2]	Orthogonal contrasts		
	0	10	20	30		Linear	Quadratic	Cubic
DMI (kg/day)	1.42	1.40	1.44	1.52	0.11	0.47	0.57	0.77
N intake (g/day)	27.80	27.44	28.08	29.50	1.78	0.45	0.70	0.81
Fecal N (g/day)	9.28	8.69	8.30	9.86	1.08	0.78	0.34	0.72
Urinary N (g/day)	0.90	1.21	0.98	1.07	0.17	0.72	0.54	0.31
N retained (g/day)	7.79	8.01	8.58	8.87	1.58	0.38	0.99	0.52

[1]0: Control diet (without silage), 10: diet containing 10% silage, 20: diet containing 20% silage and 30: diet containing 30% silage.
[2]SEM= Standard error of the mean.

Table 5. Nutrient digestibility (%) in sheep fed with the experimental diets

Items	Diets[1]				SEM[2]	Orthogonal contrasts		
	0	10	20	30		Linear	Quadratic	Cubic
Dry matter	59.49	59.50	62.86	62.48	1.65	0.12	0.90	0.36
Organic matter	61.39	61.69	65.11	64.84	1.49	0.06	0.85	0.33
Crud protein	64.65	65.28	63.61	63.76	2.34	0.95	0.27	0.32
Neutral detergent fiber	30.82	31.69	34.67	34.29	3.21	0.35	0.94	0.78

[1]0: Control diet (without silage), 10: diet containing 10% silage, 20: diet containing 20% silage and 30: diet containing 30% silage.
[2]SEM= Standard error of the mean.

The mean values for daily excretion of purine derivatives in urine and microbial protein synthesis are shown in Table 6. Allantoin, creatinine and total purine derivatives in urine changed quadratically by feeding the experimental diets ($P<0.05$). Animals receiving the control diet recorded the lowest amount of allantoin, while those receiving 20% silage had the highest values. The higher values for creatinine and total purine derivatives were detected in the control animals, whereas the animals receiving the diet with 30% silage recorded the lowest level. Uric acid and microbial protein synthesis were not affected by increasing the level of silage.

Blood glucose, urea N, triglyceride, total protein, cholesterol and total creatinine concentrations were not affected by increasing the level of silage in the diets (Table 7).

Ruminal pH and NH_3-N values were not affected by feeding experimental diet (Figure 1).

The Holotrich and cellulolytic populations in the ruminal fluid were not affected by feeding the experimental diets, but Entodinium and total protozoa species were increased linearly with increased level of alfalfa pulp ensiled with waste date (Table 8).

Discussion

Chemical composition and quality of silage

The results of the present study showed that the amount of DM was increased with ensiling alfalfa pulp

Table 6. Urinary purine derivatives and microbial protein synthesis in sheep fed with the experimental diets

Items	Diets[1]				SEM[2]	Orthogonal contrasts		
	0	10	20	30		Linear	Quadratic	Cubic
Allantoin (mmol/d)	8.21	8.61	8.70	8.48	1.89	0.06	0.02	0.03
Creatinine (mmol/d)	18.40	17.23	17.40	16.98	3.79	0.06	0.02	0.03
Uric acid (mmol/d)	0.14	0.17	0.14	0.16	1.55	0.15	0.32	0.41
Total of purine derivatives (mmol/d)	10.94	10.28	10.38	10.14	2.13	0.06	0.02	0.03
Microbial protein synthesis (g/day)	47.63	54.40	55.50	50.23	11.12	0.13	0.27	0.17

[1]0: Control diet (without silage), 10: diet containing 10% silage, 20: diet containing 20% silage and 30: diet containing 30% silage.
[2]SEM= Standard error of the mean.

Table 7. Blood parameters (mg/dL) in sheep fed with the experimental diets

Items	Diets[1]				SEM[2]	Orthogonal contrasts		
	0	10	20	30		Linear	Quadratic	Cubic
Blood glucose	66.87	72.37	68.75	80.57	6.18	0.20	0.62	0.39
Urea nitrogen	33.75	31.50	33.25	31.75	4.22	0.82	0.93	0.71
Triglyceride	21.55	20.62	22.50	22.80	2.07	0.55	0.77	0.64
Total protein	8.37	8.87	9.00	8.57	0.28	0.58	0.14	0.89
Cholesterol	44.80	43.82	46.02	44.50	2.14	0.89	0.90	0.49
Creatinine	1.06	1.04	1.04	0.95	0.05	0.25	0.56	0.62

[1]0: Control diet (without silage), 10: diet containing 10% silage, 20: diet containing 20% silage and 30: diet containing 30% silage.
[2]SEM= Standard error of mean.

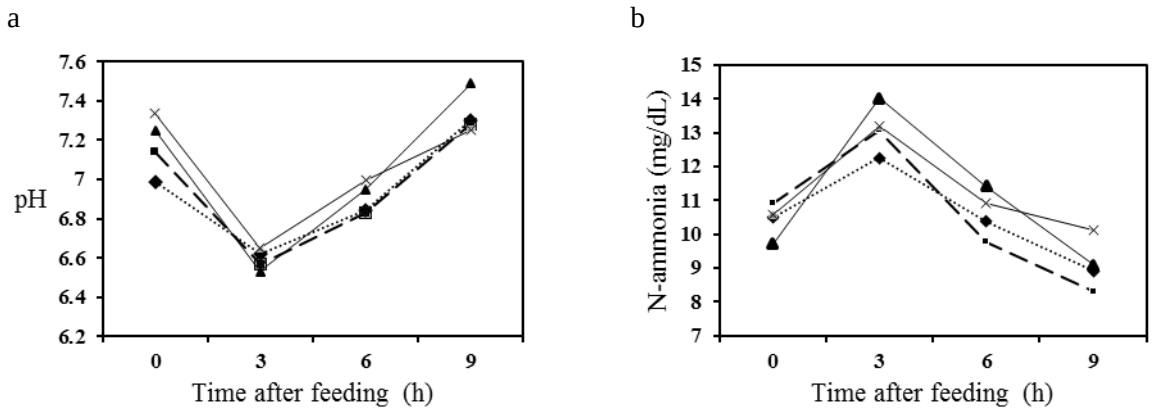

Figure 1. pH value (a) and NH3-N concentration (b) for the ruminal fluid of sheep fed treatment diets. control diet (···♦···), diet containing 10% silage(– –■– –), diet containing 20% silage (—▲—) and diet containing 30% silage (—✕—)

Table 8. Rumen protozoa population (×10^5/mL rumen liquid) in sheep fed with the experimental diets

Items	Diets[1]				SEM[2]	Orthogonal contrasts		
	0	10	20	30		Linear	Quadratic	Cubic
Holotryches	0.21	0.17	0.17	0.17	0.04	0.30	0.11	0.24
Cellulolytic	0.13	0.16	0.12	0.13	0.02	0.07	0.36	0.50
Entodinium	13.75 [b]	20.56 [a]	21.96 [a]	23.19 [a]	2.02	0.03	0.56	0.89
Total protozoa	14.09 [b]	20.89 [a]	22.26 [a]	23.50 [a]	2.1	0.03	0.54	0.74

[1]0: Control diet (without silage), 10: diet containing 10% silage, 20: diet containing 20% silage and 30: diet containing 30% silage.
[2]SEM= Standard error of mean.
[a–b] Means within a row with common superscript are not significantly different (P>0.05).

with waste date, which was probably due to high DM content in waste date. Rajabi et al. (2016) reported that adding waste date to alfalfa during ensiling increased DM and OM but decreased CP, EE, ash and NDF of silages. Ziaei (2010) observed that supplementing alhagi silage with waste date significantly decreased DM content of silage. Different levels (5, 10 and 15%) of low-quality date palm were used for ensiling *Alhagi maurorum* and it was found that silage with 15% waste date had lower DM as compared with others.

The CP level was not changed in alfalfa-pulp silage. It is reported that molasses addition can increase fermentable carbohydrates in ensiled forage and provides a rich source of the rapid-digesting carbohydrates for the microorganisms in the silage mass (Touqir et al., 2007). As a result, it accelerates their growth and proliferation and reduces the pH value. A more acidic the environment will prevent protein losses through plant enzymes (Borreani et al., 2018). Dates like molasses may act as a source of the rapid-digesting sugars, and, recompense their lower protein content compared to alfalfa pulps. It may be said that significant amounts of protein have been obtained during ensiling that compensate protein deficiency in date. Rajabi et al. (2016) used different levels of waste date in alfalfa silage and reported that CP content of alfalfa silage containing the highest level of waste date (15%) significantly decreased compared to other experimental silages, possibly decreased CP content in diets containing higher levels of waste date might be attributed to a low CP level in date as compared with alfalfa. Contrary to our results, Khalili and Huhtanen (2002) reported that the level of crude fiber was decreased in sorghum silage produced with molasses.

Due to existing waste date in silage, the Flieg point of silage was kept to a suitable level, as Guney et al. (2007) reported that the high level of fermentable carbohydrates increased lactic acid production and decreased the pH value. A study conducted by Rajabi et al. (2016) demonstrated that alfalfa silage with 15% waste date had highest Flieg point (Flieg point= 94.2), that indicating high quality of this silage as compared with other experimental silages. The pH is considered as one of the important indicators for evaluation of silage quality, which can be measured the level of lactic acid produced in the silage, and the quality of the fermentation process as well as the sustainability of silage materials (McDonald et al., 1991). In this study, a total score for alfalfa-pulp silage with waste date was obtained 19 that seemed to be a good score according to sensory evaluations. This may be due to greater supply of rapidly fermentable carbohydrates to grow lactic acid bacteria. In their study, Rajabi et al. (2016) reported that a total score for alfalfa ensiled with 15% waste date was 20, indicating a very good score.

DMI and digestibility

In the present study, the amounts of DM consumed were not affected by experimental diets. Because the experimental diets had the same NDF, the amounts of DMI were not different. Allen (2000) showed that as dietary NDF content reduced, the amount of DMI increased, which was consistent with our results. Rajabi et al. (2016) reported that the amounts of DMI were increased by feeding alfalfa silage with waste date. The waste date increased the amount of DMI in animal either by increasing ration palatability or increasing the passing rate for materials through the rumen (Khalili and Huhtanen, 2002).

In the present study, urinary and fecal N excretion did not change because of the same CP content in experimental diets and no difference in DMI. Urinary N excretion was associated the DMI and was about 7.5 g per kg DMI (Castillo et al., 2001) or 6 percent of the DMI (Van Soest, 1994). Salisbury et al. (2004) found that urinary and fecal N excretion was increased with increasing diet CP level when consuming rumen undegradable protein. In another study, Rajabi et al. (2016) found that fecal N excretion of sheep fed with experimental diets containing alfalfa silage with different levels of waste date (0, 5, 10 and 15%) is not affected, which was consistent with our results.

Increasing N balance in body led to increase animal

feed N efficiency and reduced N losses (Castillo et al., 2001). Moreover, Rihani et al. (1993) found that increased N retention may be attributed to either an increase in microbial protein yield or higher passage N obtained from ruminal degradability or both of these outcomes. Contrary to our results, Rajabi et al. (2016) reported that N intake was increased by increasing the level of waste date in experimental diets which was related to increase DMI by animals.

In this study, no significant difference was observed in digestibility of DM, OM, CP and NDF in the experimental diets. Broderick (1995) found that apparent digestibility of NDF and ADF in cows fed diets containing alfalfa silage was significantly higher than that in those fed diets containing alfalfa hay. Contrary to our results, Alhomidy et al. (2011) reported that the presence of discarded dates improved growth and efficiency of digestion in sheep. On the other hand, Al-Dobaid et al. (2009) in their study found that the apparent digestibility of CP in experimental diets was decreased by increasing the level of waste date (30%) as compared with the control diet.

Purine derivative and microbial synthesis

In the present study, the presence of alfalfa pulp ensiled with waste date in sheep diet had no effect on total purine derivatives, but allantoin, creatinine and total purine derivatives changed quadratically. In their experiment, (Khezri et al., 2016) reported a linear increasing in urinary allantoin excretion and microbial protein synthesis by increasing the level of date pulp up to 14% in sheep diet. This might be related to synchronization of rapidly fermentable carbohydrates and ruminally degradable N in the rumen (Sniffen et al., 1992).

With increasing DMI, growth and proliferation of rumen microorganisms has increased due to the availability of energy for microorganisms, leading to an increase in both allantoin and microbial protein synthesis. In the present study, urinary uric acid concentration in animals was not affected by experimental diets. Uric acid is one of the major derivatives in urine and is used to calculate purine derivatives. A positive correlation was observed between microbial protein and uric acid excretion (Johnson et al., 1998). It can be expected that uric acid will not be influenced because the microbial protein is not affected. Rajabi et al. (2016) found that lower levels of uric acid in diets containing alfalfa silage may be due to the lack of fermentable carbohydrates in the rumen and reduced efficiency of microbial protein synthesis as compared with diets containing alfalfa ensiled with 15% waste dates. In the current study, the creatinine level was not affected by experimental diets but quadratically changed ($p < 0.05$). Creatinine is a break-down product of creatine phosphate in muscle tissue. It is usually excrete at a fairly constant rate and depends on the body size (Chen et al., 1992). In this study, total purine derivatives in sheep were not affected by experimental diets. Source of urinary purine derivatives are absorbed microbial purines and purines are obtained from as animal's own tissue (Chen and Orskov, 2004).

The microbial protein synthesis was not affected by experimental diets. Due to the fact that the amounts of feed and N consumed by sheep were not affected by experimental diets; it could therefore be said that no difference was found between ammonia N and microbial protein. The energy required for the bacteria is provided with increasing the soluble carbohydrates, resulting in better use of diet protein and the ammonia produced is used by microorganisms, leading to increased production of microbial protein (Russell et al., 1992). It has been reported that microbial growth is highly determined by a synchronous supply of energy and N in the rumen (Hristov and Jouany, 2005; Salariniya et al., 2012). In the gut lumen, microbial urease is responsible for the degradation of urea to NH_3-N, and therefore recycled N can be used for microbial protein synthesis or absorbed as NH_3-N which may led improvement of N retention in animal's body (Reynolds and Kristensen, 2008).

Blood parameters

In this study, there was no statistically significant difference between serum glucose, triglyceride and cholesterol levels in sheep. Contrary to our results, Rajabi et al. (2016) showed that blood glucose levels in sheep were increased with that increasing the levels of waste date in alfalfa silage. A high concentration of the rapid-digesting carbohydrates in waste date can increase the propionate concentration in rumen fluid, leading to increased levels of blood glucose in the sheep. They also reported that triglyceride levels were not change by increasing alfalfa ensiled with waste date, but blood cholesterol increased, which might be due to increased carbohydrate and DMI. Creatinine is a breakdown product of blood protein and is related to muscle mass. In this study, blood urea N, triglyceride and total protein were not affected by experimental diets. Some studies shown that more than 60% of plasma urea-N can be obtained from absorption of ruminal ammonia (Depeters et al., 1992). In their study, Azizi-Shotokhoft

et al. (2013) reported that the levels of blood urea N are influenced by the rumen ammonia levels. However, another study showed that diet containing lower alfalfa silage versus diet containing corn silage had higher urea N levels, which might be due to lack of a synchronous supply of energy and RDP in the rumen (Brito and Broderick, 2006).

Rumen parameters

In the current study, Ruminal pH values varied from 6.5 to 7.4, which are in 6.7 ± 0.5 optimal pH ranges to maintain normal cellulolytic bacteria activity (Russell and Wilson, 1996), and above 6.0, which is required for microbial protein synthesis (Russell et al., 1992). Feeding diets containing alfalfa pulp ensiled with waste date did not affect ruminal pH in animals. Ruminal pH was not changed in sheep fed experimental diets due to the role of protozoa on pH stabilization (Douglas and Veira, 1987). In another study, Khezri et al. (2016) reported that, ruminal pH was increased with increasing levels of discarded dates in diets instead of dietary carbohydrate, because other sugars compared to starches provided less carbon for acid production (Hall and Herejk, 2001). The average concentrations of ammonia-N in the rumen were not affected by increasing levels of alfalfa-pulp ensiled with waste date in diets. It can due to both the same amount of DMI and the same CP content in the experimental diets. Rajabi et al. (2016) also reported that the concentrations of total ammonia-N in rumen of sheep were decreased by increasing the level of waste date in alfalfa silage. Reduction in ammonia-N concentrations showed a more efficient use of rapidly-fermentable parts of diet as well as enhancing growth and metabolism of ruminal microorganisms (Jakyeom et al., 2010). On the other hand Ivan et al. (2000) demonstrated that increased N concentrations in the rumen fluid may be due to the higher number of protozoa in the rumen. Protozoa exhibit a variety of proteolytic activities, which produced ammonia by the ingestion of ruminal bacteria.

In this study, the population of Holotryches in the rumen fluid of sheep fed diets containing alfalfa pulp ensiled with waste date was not affected. In their study, Rajabi et al. (2016) reported that cellulolytic population per mL rumen fluid of sheep fed diets containing alfalfa ensiled with waste date was affected. Increased cellulolytic population may be attributed to a higher supply of the rapid-digesting carbohydrates for their more growth, and improved microbial protein yield. Among protozoa species, Entodinium species had the highest numbers because it is the most predominant ruminal ciliate species, which is consistent with the findings of a study conducted by Dehority (2005) who reported that predominance of Entodinium species may be due to their higher resistance to different rumen conditions as compared with other species. In another study, Franzolin and Dehority (1996) found that the number of protozoal Entodinium spp. increased with the inclusion of concentrate in the diets containing forage.

Conclusions

In this study, the chemical composition of alfalfa pulp showed that it has relatively high levels of nutrients and that its silage can be fed as a portion of the diet of sheep. Feeding alfalfa pulp ensiled with waste date to sheep did not affect feed intake and nutrient digestibility. Due to the relatively low costs of alfalfa pulp and waste date, their inclusion in sheep diets can reduce feeding cost, overall production costs and environmental pollution.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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