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Full Length Research Paper

Determination of digestibility of almond hull in sheep

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A study was conducted to determine the chemical composition and digestibility of almond hull using *in-vitro*, *sacco* and *vivo* methods. Chemical compositions of almond hull and alfalfa hay were determined. The crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of almond hull were lower than those of alfalfa hay but the non fibrous carbohydrate (NFC) was more than that of alfalfa hay (3.5 times) ($P < 0.01$). The dry matter disappearance of almond hull for all incubation times using *in sacco* method was greater than that of alfalfa hay ($P < 0.01$). To measure *in vivo* digestibility, four mature Moghani sheep of live weight (39 ± 3 kg mean±SD) were used in a 2 × 2 change over design. Diets consisted of a basal diet (alfalfa hay) and a mixed diet (alfalfa hay 70% + almond hull 30%). *In-vitro* and *vivo* dry matter digestibilities of almond hull were 585.8 and 645.0 gkg^{-1}, respectively. There was no difference between *in vivo* dry and organic matter digestibility of alfalfa hay and mixed diet but were significantly affected in the case of CP and NDF ($P < 0.01$). In general, almond hull had low CP but greater NFC content as well as greater digestibility in sheep.

Key words: Almond hull, by-product, chemical composition, digestibility, *in vivo*.

INTRODUCTION

Almond, scientifically known as *Prunus dulcis*, belongs to the Rosaceae family and is also related to stone fruits such as peaches, plums and cherries (Jahanban et al., 2009). The United States, specifically California, is the major producer of almond (Sathe et al., 2002; Wijerante et al*.,* 2006). Almond, with or without the brown skin, is consumed with the whole nut or used in various confectioneries and chocolates; its discarded components are used as livestock feed (Takeoka et al., 2000). Almond hull by-product, are obtained by drying the portion of the almond fruit that surrounds the hard shell. The proportion of the hull, shell and nut is 50% hull, 25% shell and 25% nut on an air-dry basis (Aguilar et al., 1984; Fadel, 1999). A by-product feedstuff is a product that has value as an animal feed and is obtained during the harvesting or processing of a commodity in which human food or fiber is derived. By-product feedstuffs can be of either plant or animal origin. Growing interest in identifying and quantifying by-product feedstuffs is due to the desire to understand and monitor environmental wastes in most countries (Fadel, 1999).

The world-wide use of by-product feedstuffs is a common practice, yet few published reports document the amounts of plant by-product feedstuffs generated (Grasser et al., 1995). Production of almonds and the by-product hull has been increasing rapidly in recent years. Various workers examined the chemical composition and nutritive value of almond hull and reported that hull as a feedstuff contained 2.1 to 8% crude protein (CP) (Fadel, 1999; Getachew et al., 2002), 1.69 to 2.9% ether extract (EE) (Reed and Brown, 1988; Getachew et al., 2004), 28 to 38.49% neutral detergent fiber (NDF) (Reed and Brown, 1988; Getachew et al., 2004), 48.7 to 57.8% non fibrous carbohydrate (NFC) (Reed and Brown, 1988), 59.6 to 66.7% *in vivo* dry matter digestibility (DMD) (Alibes et al., 1983; Aguilar et al., 1984) and 1.85 to 2.87 Mcal/kg meta-

Abbreviations: DM, Dry matter; **CP,** crude protein; **EE,** ether extract; **ADF,** acid detergent fiber; **ADL,** acid detergent lignin; **NDF,** neutral detergent fiber; **NFC,** non fibrous carbohydrates; **OM,** organic matter; **DMD,** dry matter digestibility; **OMD,** organic matter digestibility; **DOMD,** digestible organic matter in dry matter; **ME,** metabolizable energy; **DCEL,** digestibility of cellulose; **DHEM,** digestibility of hemicellulose; **DCP,** digestibility of crude protein; **DNDF,** digestibility of neutral detergent fiber; **DADF,** digestibility of acid detergent fiber; **TMR,** total mixed ration.

bolizable energy (Alibes et al., 1983; Fadel, 1999). The objective of this study was to evaluate the chemical composition and digestibility of almond hull as a feed for sheep.

MATERIALS AND METHODS

The almond hull was collected from several almond gardens in the north-western part of Iran. Broken branches, leaves, dust and other residuals were separated from hulls, then chopped (about 2 cm) and mixed completely. Alfalfa hay in full bloom was cut and sun-cured, then chopped (about 2 cm) and stored on concrete in an enclosed building for use as comparison feed and basal diet in this experiment. Dry matter (DM) was determined from fresh samples in an oven at 105°C for 24 h or until it reaches a constant weight (AOAC, 1995). The residual samples were oven-dried at 55°C for 48 h. Samples of 200 g oven-dried forage from each treatment were ground in a Wiley mill (1-mm screen) and used for subsequent chemical analysis. CP, EE, Ash, acid detergent fiber (ADF) and acid detergent lignin (ADL) contents of samples were determined by standard methods (AOAC, 1995). Neutral detergent fiber (NDF) was analyzed according to Van-Soest et al. (1991). Non fibrous carbohydrate (NFC), cellulose and hemicellulose were calculated (NRC, 2001) as follows:

NFC = 100 – (CP% + NDF% + EE% + Ash%)

Cellulose = (ADF% - ADL%)

Hemicellulose = (NDF% - ADF%)

The *in sacco* method was used to determine the DM and NDF digestibility of feeds when suspended in the rumens of three rumen-fistulated Balochi wether sheep of approximately 50 ± 3 kg (mean±SD) live weight. The animals were fed 1.3 kg day^{-1} of a ration consisting of alfalfa hay, wheat straw, barley and wheat bran with a ratio of forage to concentrate of 60:40 (DM basis), which was calculated to provide maintenance requirements. Diet as total mixed ration (TMR) was given to sheep in two equal portions at 08:00 and 18:00 h. The polyester bag size for determination of DM disappearance was 12 × 19 cm, with a pore size of 50 µm. All samples were dried and milled through a 4.0-mm sieve (Spanghero et al., 2003; Yalchi and Kargar 2010). Then, 5 g of each sample was put in the nylon bags and incubated in the rumen for 2, 4, 12, 24, 48, 72 and 96 h. All bags were inserted at the same time, just before the morning feeding (08:00 h). For each sheep, one bag was used for each time interval. Upon withdrawing the bags from the rumen, they were washed in a washing machine for 1 h using cold water, and then kept in a freezer. When all bags had been taken from the rumen, they were dried for 2 days at 55°C. For each bag, the residue was analyzed for DM. To determine NDF disappearance, bag size and sample weight were 3 × 6 cm with a pore size of 46 µm and 0.5 g, respectively. For each bag, the residue was analyzed for NDF. Disappearance of DM and NDF at each incubation time was calculated from the proportion remaining after incubation in the rumen and was reported for digestibility at time.

The *in vitro* DM digestibility, organic matter (OM) digestibility and digestible OM in DM were determined according to the two-stage technique of Tilley and Terry (1963) with rumen liquor collected by stomach tube suction, aided by vacuum pump from mature Iranian Moghani sheep. These sheep with average weight of 45 ± 4 kg (mean±SD) were fed 1.25 kg alfalfa hay and 0.25 kg common concentrate (barley 60%, wheat bran 35% and minerals and vitamins supplement 5%) for each, two weeks before the beginning of the experiment and during collection period. Animals were not fed before rumen liquor was collected. The liquor was collected in a flask immersed in warm water and maintained at 39°C, strained through three layers of cheesecloth, and O_2-free CO_2 was bubbled slightly through it before dispensing into 100 ml tubes.

An *in vivo* digestibility experiment was measured using the total fecal collection method (Givens et al., 2000). Four mature Moghani sheep of live weight (39 ± 3 kg mean±SD) were used. Sheep were fasted for 12 h prior to weighing at the beginning and at the end of each experimental period. Two weeks before the start of the experiment, sheep were treated against internal parasites and supplied with an intramuscular injection of A, D_3 and E vitamins. The animals were penned in individual metabolic cages that allowed separated collection of feces. They had free-access to water and salt stone. Two diets were used: Containing basal diet (alfalfa hay) and mixed diet (alfalfa hay 70% + almond hull 30%). The diets were supplemented with the same amounts of minerals and vitamins supplement. Diets were offered simultaneously to the four animals, using a 2 × 2 change over design (two sheep for each diet in each period). Each period lasted for two weeks, of which first and subsequent week were adaptation and sampling period, respectively. The animals were offered feed at maintenance level, 0.87 ± 0.05 kg day^{-1} (Ensminger, 2002). The sheep were fed twice daily, around 08:00 and 17:00 h. Samples of feeds were taken daily for analysis of chemical composition. The faeces from each sheep on each treatment were weighed and a 10% sample was frozen at -20°C for later analysis.

Apparent digestibility coefficient of *in vivo* experiment for nutrients (such as DM, OM, CP, NDF, ADF, cellulose and hemi-cellulose) was calculated for each diet on the basis of quantitative data for intake and output (Givens et al., 2000) as follows:

Digestibility = (nutrient intake – nutrient excreted in feces) ÷ nutrient intake (1)

The digestible OM in DM (DOMD) and metabolizable energy (ME) of the feeds were calculated according to McDonald et al. (2002) as follows:

DOMD = (OM intake – OM excreted in feces) ÷ DM intake (2)

ME (MJ/kg DM) = 0.016 × DOMD (g/kg DM) (3)

Digestibility coefficients of almond hull (test feed) were calculated using difference method (assumes no associative effects) as follows (Givens et al., 2000):

TD = (BF × DB) + (TF × DT) (4)

Where, TD is digestibility of mixed diet (contained basal diet and test feed), BF is the percentage of basal diet in mixed diet, DB is digestibility of basal diet, TF is the percentage of test feed in mixed diet and DT is digestibility of test feed.

The means from chemical analysis and *in sacco* results of alfalfa hay and almond hull were compared by the t-test. Data obtained from *in vivo* digestibility were analyzed as a 2 × 2 change over design using MIXED procedure of SAS (1985).

RESULTS AND DISCUSSION

Chemical composition of alfalfa hay and almond hull are shown in Table 1. There were significant differences in chemical composition between alfalfa hay and almond hull except for DM and EE content. The DM content of almond hull was 895.0 gkg^{-1}, however Arosemena et al. (1995) reported that DM of almond hull varied from 846.8 to 894.5 with a mean of 880.2 g kg^{-1}. The EE and ash content of almond hull (16.5 and 64.7 gkg^{-1} DM) were

Table 1. Mean chemical composition of alfalfa hay and almond hull (gkg^{-1} DM) (n = 4).

Item	Alfalfa hay	Almond hull	P value
DM [a]	911.0±9.3 [b]	895.0±10.8	0.06
Ash	102.8±4.2	64.7±1.7	0.01
CP	148.0±6.1	28.6±1.4	0.01
EE	18.8±5.4	16.5±5.4	0.58
NDF	585.9±5.5	371.4±21.8	0.01
ADF	367.4±5.6	242.8±24.8	0.01
ADL	98.9±2.8	117.8±9.4	0.01
Cellulose	268.5±8.0	125.0±20.3	0.01
Hemicellulose	218.5±7.8	128.6±3.1	0.01
NFC	144.6±11.8	518.8±28.6	0.01

[a] ($g\ kg^{-1}$ as fed); [b] mean±SD; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFC: non fibrous carbohydrates.

Table 2. Dry matter and neutral fiber digestibility (%) of alfalfa hay and almond hull with *in sacco* method in different incubation times.

Incubation time	Dry matter				Neutral detergent fiber			
	Alfalfa hay	Almond hull	SE	P value	Alfalfa hay	Almond hull	SE	P value
2	23.97	47.57	1.14	0.01	8.32	1.39	0.20	0.01
4	25.35	47.76	1.22	0.01	8.22	4.68	0.84	0.01
12	36.70	55.66	0.24	0.01	16.61	10.97	0.38	0.02
24	63.33	71.47	0.38	0.01	44.54	39.32	0.77	0.02
48	64.68	71.37	0.72	0.01	50.69	42.57	1.59	0.01
72	64.77	76.27	1.56	0.01	53.72	51.29	1.02	0.10
96	66.63	77.40	0.55	0.01	52.17	55.08	1.09	0.06

SE: Standard error.

similar to previous reports (Reed and Brown, 1988; Getachew et al., 2002, 2004). A very low CP of almond hull (28.6 gkg^{-1} DM), in the current study agreed with previous report (Fadel, 1999) but not with others (NRC, 2001; Getachew et al., 2004).

Differences among studies may be related to species or genetic variation. The CP content of almond hull usually varies in the range of 38.7 and a high 80.0 $g\ kg^{-1}$ DM (Arosemena et al., 1995). However, the alfalfa hay which was used in this experiment, has been cut in full-bloom, and has medium quality and its CP content was almost 5 times more than that of almond hull.

There was a main difference between almond hull and alfalfa hay for cell wall content such as NDF, ADF or cellulose and hemicellulose (except ADL content) and NFC content. Except for NFC, the cell wall content of almond hull was lower than that of alfalfa hay. Some researchers (Depeters et al., 1997; Getachew et al., 2004) have reported that the NFC content of almond hull is 487 to 578 and 504.5 to 553.9 gkg^{-1} DM, respectively. The NFC content of almond hull was almost 3.5 times more than that of alfalfa hay. However, the acid detergent lignin (ADL) content of almond hull was greater than that of alfalfa hay. The NDF and NFC content of almond hull in this experiment were 371.4 and 518.8 gkg^{-1}, respectively, which agrees with previous reports (Reed and Brown, 1988; Getachew et al., 2004).

The DM and NDF digestibility of alfalfa hay and almond hull at different times of incubation are shown in Table 2. The DM digestibility of almond hull for all incubation times was greater than those for alfalfa hay ($P < 0.01$). This effect can be attributed to greater NFC as well as lower NDF and ADF content of almond hull when compared to alfalfa hay (Table 1). Greater disappearance rate of almond hull when compared to alfalfa hay at 2 and 4 h post incubation times (47.57 and 47.76 vs. 23.97 and 25.35, respectively) might increase voluntary DM intake (McDonald et al., 2002). In contrast to Shultz et al. (1993), disappearance rate after 24-h incubation was greater than that reported by them (71.47 vs. 56%). Except for 72-h incubation, the NDF digestibility of almond hull was lower at each incubation time ($P < 0.05$).

Table 3. Mean digestibility and metabolizable energy of almond hull (DM basis) (n = 4).

Item	*In vitro*	*In vivo* [a]
DMD (g kg^{-1})	585.8±26.8 [b]	645.0±65.8
OMD (g kg^{-1})	530.8±23.9	640.3±59.0
DOMD (g kg^{-1})	506.3±22.8	598.8±52.8
DCP (g kg^{-1})	ND	282.8±86.1
ME (MJ kg^{-1})	ND	9.59±0.86 [c]

[a]Calculated from equation 4; [b] mean±SD; [c]calculated from equation 3; DMD, dry matter digestibility; OMD, organic matter digestibility; DOMD, digestible organic matter in dry matter; DCP, digestible crude protein; ME, metabolizable energy; ND, not data.

Table 4. Mean *in vivo* digestibility and ME of diets (gkg^{-1} DM).

Item	Basal diet [a]	Mixed diet [b]	P value	SE
DMD	643.4	643.9	0.97	9.53
OMD	658.7	653.2	0.60	8.47
DOMD	588.8	591.8	0.81	7.58
DCP	662.7	542.7	0.01	9.59
DNDF	601.1	504.5	0.01	8.63
DADF	571.1	444.1	0.07	9.06
DCEL	681.8	611.5	0.16	24.62
DHEM	651.5	608.1	0.06	8.39
ME (MJ kg^{-1})	9.42	9.44	0.93	1.25

[a]Containing alfalfa hay; [b] containing alfalfa hay 70% + almond hull 30%; SE, standard error; DMD, dry matter digestibility; OMD, organic matter digestibility; DOMD, digestible organic matter in dry matter; DCP, digestibility of crude protein; DNDF, digestibility of neutral detergent fiber; DADF, digestibility of acid detergent fiber; DCEL, digestibility of cellulose; DHEM, digestibility of hemicellulose; ME, metabolizable energy.

Varga (2006) reported that readily available non-forage fiber sources such as almond hull, can affect fiber digestibility of diet. The NDF digestibility is a function of the potentially digestible fraction and its digestion and passage rates. *In vivo* NDF digestibility is confounded by different retention times in the rumen, which can be attributed to differences in DM intake (Oba and Allen, 1999). In addition, exposure to acidic conditions in the small intestine and fermentation in the large intestine (*in vivo* experiment) might reduce differences observed for fermentation by rumen microorganisms (*in situ* experiment). For this reason, NDF digestibility determined *in situ* is an important measure of forage quality and should be distinguished from NDF digestibility *in vivo* (Varga, 2006).

Mean *in- vitro* and *vivo* digestibility coefficients (without statistically analysis between them) of almond hull are shown in Table 3. Results showed that *in vivo* DMD, OMD and DOMD of almond hull were approximately 10.1, 17.1 and 15.5% units greater than *in vitro* study, respectively. Dry matter digestibility of almond hull was calculated as 645.0 g kg^{-1} DM, which agrees with previous report (Alibes et al., 1983). However, DMD of almond hull in current study was greater than that observed by Aguilar et al. (1984) (645 vs. 596 g kg^{-1} DM). Calculated ME content of almond hull (9.59 MJ kg^{-1} or 2.29 Mcal kg^{-1}) was in agreement with others (Alibes et al., 1983; Aguilar et al., 1984; NRC, 2001).

Mean *in vivo* digestibility coefficients and ME of diets containing alfalfa hay and mixed diet (alfalfa hay 70% and almond hull 30%) are shown in Table 4. There was no difference between basal and mixed diet on DM, OM, OMD, ME, cellulose and hemicellulose digestibilities, except for CP (DCP), NDF (DNDF) and ADF (DADF). The chemical composition of diets affects digestibility of nutrients. Results of the current study showed that there is substantial difference between almond hull and alfalfa hay for CP and cell wall content as it was obvious for digestibility coefficients of nutrients. There was no significant difference between basal and mixed diet for DOMD, thus no difference was observed for ME. Almond hull in comparison with alfalfa hay had greater NFC and lower NDF content, thus this feedstuff might cause lower chewing and rumination activity and greater passage rate in digestion tract of sheep (McDonald et al., 2002). Also, ADL content of almond hull is greater when compared to alfalfa hay (Table 1). Therefore, lower NDF and ADF digestibility of mixed diet may be attributed to this. Furthermore, almond hull CP content is very low (28.6 gkg^{-1}) and usually low CP content diets reduce micro-organisms growth in rumen, and cause limitation of fiber digestion (McDonald et al., 2002). Crude protein requirements of sheep is estimated to be 94 to 150 gkg^{-1} of dietary DM (Ensminger, 2002) and therefore CP concentration of the almond hull used in this experiment would be inadequate for providing maintenance and growing requirements of sheep (Ensminger, 2002). So, treating almond hull with urea as a cheap resource on nitrogen is recommended (Yalchi, 2010).

Conclusion

Almond hull had lower CP but greater NFC content when compared with alfalfa hay. Also, DMD, OMD, DOMD and ME of almond hull was similar to that full-bloom alfalfa hay. Based on *in sacco* measurement, almond hull showed greater and lower DM and NDF disappearance rate, respectively. Almond hull can be used as diets for feeding sheep during off season periods.

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