Indian J. Anim. Res., 52 (3) 2018 : 392-396
Print ISSN:0367-6722 / Online ISSN:0976-0555

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.ijaronline.in

Comparison of microbial protein synthesis and nutrient digestibility of *Medicago sativa* and *Prangos pabularia* hay in sheep

Mehtap Guney*, Sibel Erdogan[1], Mehmet Akif Karsli[2] and Murat Demirel[1]

Department of Animal Nutrition,
Faculty of Veterinary Medicine, Yuzuncu Yil University, Van, Turkey.
Received: 04-07-2016 Accepted: 12-10-2016 DOI: 10.18805/ijar.v0iOF.6986

ABSTRACT

The aim of this study was to compare feed intake, nutrients digestibility, protein fractions entering into the duodenum, and ruminal fermentation parameters of sheep fed *Medicago sativa* (alfalfa) and *Prangos pabularia,* locally called kerkol hays. In the study three ruminally and duodenally cannulated Morkaraman sheep were used. It was carried out as two periods within a 2x3 crossover experimental design. Daily intake of crude protein (CP) was higher (P<0.05) in sheep fed alfalfa than those fed kerkol hay. Digestibility of NDF and ADF were lower (P<0.05) in sheep fed alfalfa hay than those fed kerkol hay (P<0.05). The percentage of protein escaping ruminal degradation was higher (P<0.05) in sheep fed kerkol hay (30.73%) than those fed alfalfa hay (15.05%). Ruminal fermentation parameters were similar between groups, except ruminal NH_3-N concentration. It is concluded that kerkol hay can be used as forage for sheep feeding.

Key words: Alfalfa hay, Digestibility, Microbial protein synthesis, *Prangos pabularia*, Sheep.

INTRODUCTION

Utilization of locally available feed resources is one of the important strategy to reduce the increasing cost of livestock production. *Prangos pabularia* forage which is locally known as kerkol is available at low cost, provides good quality forage for sheep reared at high altitude of Indian, Russia, Iran, and Turkey (Razavi, 2012). It is a perennial plant which grows between 780-3000 m altitude on land with rocky and calcareous slopes. The entire plant is consumed by ruminants, especially sheep, during the winter as hay (Hakan *et al*., 2009). The stem and leaves of *Prangos pabularia* are green and have an aromatic odor. The plant stems can reach up to one meter, and its leaves grow to 30-45 centimeters (Sharma *et al*., 2013).

Therefore the present experiment conducted to study the comparative feeding value, nutrient digestibility, protein fractions entering into the duodenum and ruminal fermentation parameter of kerkol and alfalfa hay in sheep.

MATERIALS AND METHODS

Animal research procedures were conducted with the approval of the Local Animal Ethics Committee of Yuzuncu Yil University in Van, Turkey (Decision No. 2010/11). A rubber cannula in the rumen and a closed t-type cannula in the proximal duodenum of three Morkaraman sheep were fitted by surgical methods as described by Dougherty, (1981) and Komarek, (1981). The experiment was carried out as a 2x3 crossover experimental design. During the experiments, the sheep were placed in individual cages and were housed in an animal house with free access to clean water, vitamin and mineral blocks. Sheep were also treated for internal and external parasite just before the experiment started. Alfalfa and *Prangos pabularia* hays were obtained from forage market at Van city center.

Experiment completed in two phases each of 16 duration including ten days adaptation and six days sampling. Animals were fed twice a day at twelve-hour intervals (at 08:00 am and 20:00 hr) ad libitum level.

The amount of left-over fodder was collected at the end of 5th day of experiment to determine feed intake. Daily feed intake was calculated by subtracting the amount left-over (orts) from the amount of feed consumed at the end of the fifth day of sample collection. Forage consumed and left-over for each animal was weighed at the end of each period, sub-samples were collected afterward, and chemical composition of forage sample and leftover samples was determined to calculate daily nutrient intake.

Chromium mordanted fiber was used to determine duodenal and fecal DM flow (Russell *et al*., 1993). One gram of chromium-mordanted fiber containing approximately 2% Cr was inserted into the rumen at 08:00 am and 20:00 hr from days 3 to 16 of each period. Duodenal digesta and rectal fecal samples were collected four times daily from days 14 to 16 to determine forage digestibility and microbial protein synthesis. Each animal was weighed at the beginning and end of each period to detect live weight changes.

*Corresponding author's e-mail: mguney@yyu.edu.tr
[1]Department of Animal Nutrition, Agricultural Faculty, Yuzuncu Yil University, Van, Turkey.
[2]Department of Animal Nutrition, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Turkey.

Rumen fluid was collected at 0 2, 4, 6, 8 and 10 hours post-feeding at the last day of each period to determine ruminal ammonia nitrogen (NH_3-N) levels and pH measurements. pH values of the rumen were immediately measured with a pH-meter. Approximately, 10 ml rumen fluid was sub-sampled into a tube containing 1 ml solution of HCl (v/v) and then stored at -20 °C in the freezer until analysis (Markham, 1942).

Approximately, 2 L of rumen fluid was simultaneously collected from each animal during rumen fluid collection hours to isolate ruminal bacteria. Collected rumen fluids were composited for each animal/each period in glass jars to isolate rumen microbes using the differential centrifugation technique as described by Adamu *et al*. (1989) for microbial purine analysis and stored at -20 °C until analysis (Zinn and Owens, 1986). Oven dried samples of hays, orts, feces, and duodenum were ground to pass through a 1mm screen and then, analyzed for dry matter (DM), ash, organic matter, crude protein (AOAC, 1990), neutral detergent fibers (NDF) and acid detergent fiber (ADF) using ANKOM® fiber analyzer according to the method of Van Soest and Robertson (1985).

Duodenal digesta and isolated ruminal bacterial samples were assayed to determine purine concentration, according to Zinn and Owens (1986). Ammonia-N levels in rumen fluid and wet duodenum samples were determined using the distillation method (Nursoy, 2000). To determine amounts of chromium in fecal, duodenal and mordanted fiber samples, chromium was first extracted from samples, then analyzed by atomic absorption spectrophotometry as described by Williams *et al*. (1962).

Volatile fatty acid (VFA) concentration of rumen fluid was analyzed using a Schimadzu liquid chromatographic chain equipped with a UV detector and auto sampler/injector as described by Peu *et al*. (2004).

Amounts and co-efficiencies of apparent and true ruminal digestion, total tract digestion of nutrients and amount of total and fractions of CP passing into the duodenum were calculated according to Karsli (1998).

The amounts and percentages of nitrogen (CP) and OM truly digested in the rumen were calculated by correcting the amounts of nitrogen and OM apparently digested in the rumen for microbial nitrogen and OM as determined by the ratio of two components with purines in rumen bacterial pellet. Tota tract digestibility were calculated as total amounts and perentages of nutrient digested in both rumen and intestines of animals.

The data were subjected to independent sample t-tests that compared the difference in the means of the two groups. The analysis was performed using the PROC TTEST option in SAS (Version 9.4) packaged software (SAS, 2014).

RESULTS AND DISCUSSION

The chemical composition of the alfalfa and kerkol hay used in the study are given in Table 1. There was a significant ($P<0.05$) difference between alfalfa and kerkol hay in terms of OM, Ash, CP, and ADF concentrations, except for DM and NDF ($P<0.05$). The OM and CP contents were higher whereas ash and ADF contents of alfalfa were lower than those of kerkol and the results were in agreement to the values reported by Karsli and Russell (2000) and Kalamak *et al*. (2005). The ADF content of kerkol in the study was higher than values reported by Hakan *et al*. (2009) and be due to different harvested time of the hays.

Daily nutrient intake data revealed (Table 2) similar DM, OM, ADF and NDF intake between two groups whereas daily CP intake was significantly higher ($P<0.05$) in sheep fed alfalfa hay than in sheep fed kerkol hay. The difference in CP consumption is due to higher CP content and higher intake of alfalfa hay than kerkol hay. Similar values has been reported by Yildiz (2001) in sheep fed alfalfa hay.

Digestibility of NDF and ADF were significantly higher ($P<0.05$) in the sheep fed kerkol hay than in the alfalfa hay (Table 3). Whereas digestibility of other nutrient were similar in both the groups. Present results of DM digestion were lower than those of Al-Saiady *et al*. (2010), Karsli and Russell (1999) and in agreement with the study of Ramos *et al*. (2009). Crude Protein digestibility of feedstuffs in the rumen and total tract greatly differs depanding on many factors such as protein structure of feedstuff, exsisting of anti-nutritional factor in the feedstuff and composition of diet etc. This low ruminal CP digestibility in kerkol hay may have resulted from its protein structure (NRC, 1985).

Table1. Chemical compositions of alfalfa and kerkol hay (DM %)

Nutrition matter	Alfalfa hay		Kerkol (*Prangos ssp.*) hay		P value
	Mean	SEM	Mean	SEM	
DM	90.34	0.36	90.50	0.38	0.78
OM	92.09	0.05	91.48	0.06	0.01
Ash	7.91	0.05	8.52	0.06	0.01
CP	14.81	0.52	10.13	0.23	0.01
NDF	44.77	1.30	48.93	0.99	0.06
ADF	27.87	0.95	30.87	0.29	0.04

DM: dry matter; OM: organic matter; Ash: crude ash; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre

Table 2: Daily nutrient intakes of sheep fed alfalfa hay and kerkol

g/d	Alfalfa hay	Kerkol (*Prangos ssp.*) hay	P value
DM	996.80±67.44	874.62±30.56	0.17
OM	822.19±55.52	718.16±27.81	0.17
CP	134.72±9.70	80.78±4.38	0.01
NDF	441.51±34.83	425.27±26.15	0.73
ADF	274.16±22.65	268.05±13.87	0.83
% of body weight			
DM	2.56±0.23	2.22±0.22	0.36
OM	2.11±0.20	1.82±1.19	0.36

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre

The amounts of total CP, total microbial CP, total NH_3-N, and by-pass protein in sheep fed with alfalfa and kerkol hay were similar (Table 4). The percentage of microbial CP passing into the duodenum was lower ($P<0.05$) in sheep fed kerkol hay than alfalfa hay, whereas the percentages by-pass protein entering into the duodenum was higher ($P<0.05$) for kerkol than alfalfa hay.

This was thought to be caused by the difference between the rumen passage rate of feed, as well as by better usage of N produced by rumen microbe in sheep consumed alfalfa hay because the amount of DM and CP consumed in alfalfa hay was higher than in kerkol hay. NH_3-N flowing into duodenum in alfalfa hay fed sheep was similar to the values reported by Karsli and Russell (1999). Microbial protein synthesis in the rumen was affected by the amount and source of protein and carbohydrate consumed and their rate of degradation in the rumen (Karsli, 1998).

The values obtained for MPSE for alfalfa hay was similar to that reported by Karsli and Russell (1999). It has been reported that the efficiency of microbial protein synthesis was related to true OM digested in the rumen (Karsli and Russell, 1999).

Table 3: Nutrient digestibilities (%) of alfalfa and kerkol hays fed to sheep

Item	Alfalfa hay	SEM	Kerkol (*Prangos ssp.*) hay	SEM	P value
Dry matter					
Apparent ruminal	50.41	3.90	57.17	0.51	0.92
True ruminal	77.10	1.05	78.61	2.03	0.55
Apparent total tract	55.47	3.96	62.86	1.59	0.16
Digestion coefficient, % of intake					
Organic matter					
True ruminal	81.12	0.76	83.78	1.92	0.27
Apparent total tract	50.82	4.42	60.82	2.17	0.11
% CP digestion of intake					
Apparent ruminal	28.92	9.40	10.80	0.47	0.13
True ruminal	87.60	5.82	66.88	1.08	0.03
Apparent total tract	60.98	2.60	55.19	0.47	0.09
% NDF digestion of intake					
Apparent ruminal	31.59	7.42	54.33	2.22	0.06
% ADF digestion of intake					
Apparent ruminal	27.55	7.84	56.35	2.31	0.09
Apparent total tract	48.99	7.87	69.46	5.08	0.02

Table 4: Duodenal N flow and efficiency of microbial protein synthesis in sheep fed alfalfa and kerkol hays

Item	Alfalfa	SEM	Kerkol (*Prangos ssp.*) hay	SEM	P value
N flow to duodenum, g/d					
Total-N (CP)	103.37	13.93	87.14	3.82	0.32
Microbial CP	86.22	10.94	59.35	2.30	0.07
Total NH_3	1.24	0.22	0.94	0.13	0.31
By-pass	15.90	6.51	26.85	2.30	0.19
Duodenal N flow and efficiency, %					
Microbial CP	83.75	5.21	68.17	1.56	0.04
NH_3-N	1.20	0.13	1.09	0.19	0.67
By-pass	15.05	5.08	30.73	1.73	0.04
Efficiency of microbial protein synthesis					
(MOEFF), g MCP/100g OMTDR	13.54	2.61	10.32	0.59	0.30

MCP: Microbial CP; OMTDR: Organic matter truly digested in the rumen

Rumen fermentation parameters were similar in both the groups except NH_3-N level at two hours post-feeding (Table 5). The rumen pH values were within the recommended physiological limits for sheep fed both hays (Ergun *et al*., 2004), and the changes in rumen pH post-feeding was similar to many other studies in the literature (Ramos *et al*. 2009; Behgar *et al*.,2011).

Acetic, propionic and butyric acid concentrations were low at 0 h and steadily increased after feeding. This was due to carbohydrate digestion during the post-feeding period. The values acquired were similar to those in other studies conducted with forage sources (Doran *et al*., 2007; Ramos *et al*., 2009; Ghasemi *et al*., 2012).

Based upon the results it can be concluded that kerkol hay can be used in sheep feeding due to its nutrient composition, daily nutrient intake, digestibility and rumen fermentation characteristics.

Table 5: Post-feeding rumen fermentation parameters of sheep fed alfalfa and kerkol hays (N:18)

Rumen fermentation parameter	Alfalfa hay	SEM	Kerkol (*Prangos ssp.*) hay	SEM	P value
pH					
0 h	7.36	0.06	7.24	0.03	0.14
2 h	6.82	0.29	6.70	0.18	0.74
4 h	6.29	0.09	6.25	0.08	0.74
6 h	6.85	0.08	6.61	0.23	0.39
8 h	6.87	0.07	6.68	0.21	0.43
10 h	6.81	0.09	6.74	0.21	0.78
NH_3-N mg/dL					
0 h	12.04	0.85	13.06	0.21	0.31
2 h	18.19	0.65	10.64	0.63	0.01
4 h	10.11	1.13	7.02	0.53	0.07
6 h	6.49	0.26	6.44	0.83	0.96
8 h	6.75	0.19	7.04	1.04	0.79
10 h	9.88	1.86	6.45	1.62	0.23

NH_3-N: Ammonia; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid

Table 5 continue

Rumen fermentation parameter	Alfalfa hay	SEM	Kerkol (*Prangos ssp.*)hay	SEM	P value
Acetic acid (AA), mg/ dL					
0 h	33.87	5.82	36.27	4.88	0.77
2 h	47.75	2.03	43.63	6.64	0.59
4 h	42.58	0.56	50.65	5.36	0.21
6 h	45.34	3.93	51.71	2.25	0.23
8 h	38.83	1.96	47.74	7.28	0.30
10 h	45.15	5.36	48.91	8.53	0.73
Propionic acid (PA), mg/ dL					
0 h	6.38	0.86	8.69	1.40	0.23
2 h	13.96	1.30	12.10	0.82	0.29
4 h	12.36	1.79	12.60	1.54	0.92
6 h	13.52	1.80	13.43	1.20	0.97
8 h	11.75	0.38	10.28	1.80	0.47
10 h	12.35	1.77	10.41	2.87	0.60
Butyric acid (BA), mg/ dL					
0 h	4.92	0.86	5.22	0.61	0.79
2 h	6.93	0.10	6.05	0.63	0.24
4 h	5.67	0.47	6.23	0.79	0.57
6 h	6.52	0.82	7.23	0.50	0.50
8 h	6.50	0.29	5.79	0.28	0.16
10 h	6.50	0.67	5.97	0.52	0.56

NH_3-N: Ammonia; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid

REFERENCES

Adamu, A. M., Russell, J. R., Mcgilliard, A. D. and Trenkle, A. (1989). Effects of added dietary urea on the utilization of maize stover silage by growing beef cattle. *Anim. Feed Sci. and Tech.* **22:** 227-236.

Al-Saiady, M. Y., Abouheif, M. A., Makkawi, A. A., Ibrahim, H. A. and Al-Owaimer, A. N. (2010). Impact of particle length of alfalfa hay in the diet of growing lambs on performance, digestion and carcass characteristics. *Asian-Australas. J of Anim. Sci.* **23:** 475-482.

AOAC (1990). Association of official analytical chemists. Official methods of analysis, 15th Edition, AOAC, Washington, DC, **1:** 69-79.

Behgar, M., Valizadeh, R., Mirzaee, M., Naserian, A. A. and Ghasemi, S. (2011). The impact of alfalfa hay particle size on the utilization of soy hull by early lactating dairy cows. *Livestock Sci.* **142:** 147–154.

Doran, M. P., Laca, E. A. and Sainz, R. D. (2007). Total tract and rumen digestibility of mulberry foliage (Morus alba), alfalfa hay and oat hay in sheep. *Animal Feed Science and Tech.* **138:** 239-253.

Dougherty, R. W. (1981). Experimental surgery in farm animals. 1th (ed.) Ames, Iowa.

Ergun, A., Tuncer, S. D., Colpan, I., Yalcin, S., Yildiz, G., Kucukersan, M. K., Kucukersan, S. and Sehu, A. (2004). Hayvan Besleme ve Beslenme Hastaliklari. Veterinary Journal of Ankara University, Hayvan Besleme ve Beslenme Hastaliklari Anabilim Dali. 687p., Ankara, Turkiye.

Ghasemi, S., Naserian, A. A., Valizadeh, R., Vakili, A. R., Behgar, M., Tahmasebi, A. M. and Ghovvati, S. (2012). Partial and total substitution of alfalfa hay by pistachio byproduct modulated the counts of selected cellulolytic ruminal bacteria attached to alfalfa hay in sheep. *Livestock Sci.* **150:** 342–348.

Hakan, B., Ulker, H. and Demirel, M. (2009). Van ve çevresinde parzük, kerkol, heliz'in hayvan yemlemede kullanimi. In: VI. Nation Zootecnia Science Congress. Ataturk University. Erzurum, Turkiye.

Kalamak, A., Canbolat, O., Gurbuz, Y. and Ozay, O. (2005). Prediction of dry matter intake and dry matter digestibilities of some forages using the gas production technique in sheep. *Turkish J of Veterinary and Animal Sci.* **29:** 517-523.

Karsli, M. A. (1998). Ruminal microbial protein synthesis in sheep fed forages of varying nutritive value. Thesis (PhD.). Iowa State University. Ames Iowa, USA.

Karsli, M. A. and Russell, J. R. (1999). Ruminal microbial protein synthesis in sheep fed forages of varying nutritive values. Leaflet R1638, Iowa State University, AS.

Karsli, M. A. and Russell, J. R. (2000). Effects of source and concentrations of nitrogen and carbohydrate on ruminal microbial protein synthesis. *Turkish J of Veterinary and Animal Sci.* **26:** 201-207.

Komarek, R. J. (1981). Intestinal cannulation of cattle and sheep with a T-shaped cannula designed for total digesta collection without externalizing digesta flow. *J of Anim. Sci.* **46:** 489-503.

Markham, P. (1942). A steam distilation apparatus suitable for micro-kjeldahl analyses. *Journal Biochemistry.* **36:** 790-797.

NRC (1985). Nutrient Requirements of Sheep. Sixth revised edition. Washington, D.C., National Academy Press.

Nursoy, H. (2003). The Effects of Baker's Yeast (Saccharomyces cerevisiae) in dairy cow diets on milk yield, some rumen fluid parameters and blood metabolites of dairy cow diets. *Turkish J of Veterinary and Animal Sci.* **27:** 7-13.

Peu, P., Beline, F. and Martinez, J. (2004). Volatile fatty acids analysis from pig slurry using high-performance liquid chromatography. *International J of Environmental Analytical Chemistry.* **84:** 1017–1022.

Ramos, S., Tejido, M. L., Martínez, M. E., Ranilla, M. J. and Carro, M. D. (2009). Microbial protein synthesis, ruminal digestion, microbial populations, and nitrogen balance in sheep fed diets varying in forage-to-concentrate ratio and type of forage. *J of Anim. Sci.* **87:** 2924-2934.

Razavi, S. M. (2012). Chemical and allelopathic analyses of essential oils of Prangos pabularia Lindl. from Iran. *Natural Product Res.* **26:** 2148–2151.

Russell, J. R. (1999). Evaluation of the nitrogen and energy utilization of legume forages by growing cattle and sheep. Leopold Center Progress Report. 71-75.

Russell, J. B., Brasche, M. R. and Cowen, A. M. (1993). Effects of grazing allowance and system on the use of corn crop residues by gestating beef cows. *J of Anim. Sci.* **1:** 1256-1265.

SAS (2014). SAS/STAT Software: Hangen and Enhanced, Version 9.4, SAS, Inst. Inc., Cary, N.C. USA

Sharma, N., Negi, A. and Ashok, P. K. (2013). Standardization and phytochemical evaluation of the aerial parts of Prangos pabularia. *Journal of Pharmacognosy and Phytochemistry.* **1:** 47-50.

Van Soest, P. J. and Robertson, J. B. (1979). Systems of analyses for evaluation of fibrous feed. In: "Proc. Int. Workshop on standardization of analytical methodology for feeds" by Pigden, W. J., Balch, C.C., Graham M. (ed), Int. Dev. Res. Center. Ottowa.

Williams, C. H., David, D. H. and Iisamaa, O. I. (1962). The Determination of chromic oxide in feces samples by atomic asorbtion spectrophometry. *J of Agricultural Sci.* **19:** 381-385.

Yildiz, G. (2001). Koyunlarda bazi rumen metabolitleri ve protozoonlari üzerine degisik rasyonlarin etkisi. *Veterinary J. of Ankara Univ.* **48:** 153-158.

Zinn, R. A. and Owens, F. N. (1986). A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. *Can. J. of Anim. Sci.* **66:** 157-166.