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Dietary Supplementation Effect of Alfalfa and *Prangos pabularia* Hay on Feed Intake, Growth, and Nutrient Degradability in Kari Sheep

Muhammad Mobashar[1], Qazi Aqeel[1], Muhammad Tahir Khan[1], Assar Ali Shah[2]*, Ahmed A.A. Abdel-Wareth[3], Salman Khan[4], Nazir Ahmad[1], Sami Ullah[1] and Haq Amanullah[5]

[1]*Department of Animal Nutrition, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Peshawar 25130, Pakistan.*
[2]*Institute of Animal Sciences, Jiangsu Academy of Agriculture Sciences, Nanjing 210023, P. R. China.*
[3]*Department of Animal and Poultry Production, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt.*
[4]*School of Life Sciences, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, 210023, P. R. China.*
[5]*College of Veterinary Sciences, The University of Agriculture Peshawar, Peshawar 25130, Pakistan*

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MM, QA and AAS conceived and designed the experiments. MM, QA, MTK, SU and HA performed the experiments. MM and QA analyzed the data. AAAAW and AAS wrote the manuscript.

Key words
Feed intake, Growth performance, *In-vitro* dry matter digestibility, *In-sacco* degradability, Kari sheep

ABSTRACT

The aim of the study was to evaluate the dietary supplementation effect of alfalfa and *Prangos pabularia* hay on feed intake, growth performance, *in-vivo* and *in-saco* nutrient degradability in Kari sheep. A total of 36 Kari sheep were randomly weighed and offered the following four type of ration: A, total mix ration (TMR) + alfalfa (AL) 70 % and *Prangos pabularia* (PP) 0 % (control); B, TMR + 24% PP: 46 % AL; C, TMR + 46% PP 24 % AL and D, TMR + 70% PP: AL 0 % on dry matter basis were fed to experimental sheep for a period of 90 days. The dry matter intake, crude protein intake and organic matter intake (g/day) were significantly ($P<0.05$) affected in treatment groups. *In-vivo* nutrient digestibility, dry matter, organic matter, crude protein, crude fat, nitrogen-free extract (NFE), ash were significantly higher ($P<0.05$) in treatment groups, while in *in-sacco* degradability the DM and OM degradability at different time intervals (2h, 4h, 8h, 12h, 18h and 24 h) were significantly ($P<0.05$) higher in ration C and D. The initial body weight was significantly improved in treatment groups compared to control, while daily intake, average daily gain, and feed conversion efficiency were not affected. It is concluded that the dietary supplementation of alfalfa and *Prangos pabularia* hay increase the dry matter intake, digestibility and growth performance of Kari sheep.

INTRODUCTION

In the tropical and subtropical areas, there is a deficiency of green feed for ruminants, as well as the stream required to develop it, so there must be a way to find another resource to cover the shortfall among the conventional fodder and requirement of the animals feed. However, different feed resources available to enhance livestock production in tropical and subtropical areas are crops, fruits, foliage, grasses, by-products, shrubs, and tree (El-Waziry *et al*., 2018). Alfalfa hay is changeable in digestibility and intake, even if the harvest intended for homogeneous development failed to find a significant affiliation among the dry matter intake (DMI), and cell wall constituents of grasses and legumes (Palmonari *et al*., 2014). Alfalfa (*Medicago sativa* L.) is a perennial legume with a unique anatomy comprising moderately distinct protein-containing vegetation and fibrous stems. The maturity influences both fiber digestibility and protein fractions in alfalfa through rising the leaf and stem ratio and increasing lignifications of stems, which, in turn, alters fiber digestibility (Palmonari *et al*., 2014; El-Waziry *et al*., 2018).

The exploitation of locally offered feed resources is one of the significant strategies to reduce the rising cost of farm animals. *Prangos pabularia* locally known as kerkol is a low cost, high-quality forage for sheep reared

* Corresponding author: assaralishah@yahoo.com
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at the high altitude of India, Russia, Iran, Turkey and Pakistan (Razavi, 2012). It grows at an altitude 780-3000 m with rocky and calcareous slopes. The whole plant or hay is consumed by ruminants, particularly sheep, during the winter (Ekinci *et al*., 2018; Banday and Mir, 2012). The stem and leaves of *P. pabularia* are green and have a perfumed odor. The plant stems can achieve up to 1 m and its leaves grow to 30- 45 cm (Sharma *et al*., 2013).

The *Prangos* species have medicinal value such as carminative, tonic, anthelmintic, and anti-microbial agents. The leaves of this forage contain fundamental oil (2%), α-pinene (4%), borneol, dihydrocuminol and acetic acid derivations (17.5%). On a dry matter basis, leaves contain protein (10.4%), fat (3.5%) and fiber (22.6%) (Sharma *et al*., 2013). The crude protein digestibility of feedstuffs in the rumen and total tract greatly differs depending on various factors such as type of protein, existence of anti-nutritional factor and composition of the diet. Keeping the nutritional significance of *P. pablaria* into view, the present study was conducted to evaluate its impact on feed intake, growth performance and digestibility in Kari sheep.

MATERIALS AND METHODS

Experimental animal and design

The study was carried out in Completely Randomized Design at Small Ruminant Nutrition Research Centre, Department of Animal Nutrition, the University of Agriculture Peshawar, Pakistan. A total of 36 Kari sheep with 3 replicates with similar age and body weight were randomly distributed in 9 metabolic cages of 2.1 m × 1.05 m, with a floor mesh of 20 mm. The experimental animals were fed on 4 different rations for a period of 90 days including the adaptation period of 15 days. The experimental rations were formulated according to NRC (1985). Rations A, B, C, and D consisted of 0:70, 24:46, 46:24 and 70:0% of alfalfa and *P. pabularia* hay on a DM basis, respectively. The experimental animals were fed on each ration twice a day at 8 AM and 4 PM. Feed consumed and refused was daily recorded. Fecal sampls were collected after 24 h and body weights recorded after every 14 days. Prior to the trial, metabolic cages and all the equipment like pens, feeding areas and buckets were properly cleaned and washed with detergent. Ingredients composition of experimental rations supplemented with different levels of alfalfa and *P. pabularia* hay is given in Table I. The chemical composition of alfalfa and *P. pabularia* hay (DM basis) is given in Table II.

Alfalfa and *P. pabularia* harvested during the month of May and June, 2019 from the pasture area of District Chitral, Khyber Pakhtunkhwa (KPK), Pakistan were left in the field for drying and turned over from time to time to avoid fungal growth. The dried forage was chopped into small pieces by using the chopping machine and shifted to the experimental station of the university.

Table I. Ingredients of experimental feed containing different levels of alfalfa and *Prangos pabularia* hay.

Ingredients (g/kg)	Rations			
	A	B	C	D
Prangos pabularia hay	0.0	240.8	461.5	702.3
Alfalfa hay	697.7	440.8	230.0	0.0
Cotton seed cake	33.4	39.0	46.3	37.1
Molasses	89.0	89.6	89.5	89.8
Maize grain	55.1	58.0	58.1	48.5
Rice polish	53.9	56.9	53.1	47.4
Wheat bran	53.3	57.5	54.9	57.5
Salt	8.9	8.9	8.9	8.9
Mineral mix	8.6	8.6	8.6	8.6
Total	1000.0	1000.0	1000.0	1000.0

Rations were formulated with different levels of alfalfa and *Prangos pabularia* hay A, 0:70; B, 24:46%; C, 46:24% and D, 70:0%.

Table II. Chemical composition (%) of alfalafa and *Prangos pabularia* hay (DM basis).

Items	*Prangos pabularia*	Alfalfa
DM	92.2	91.2
CP	16.5	16.1
CF	28.1	30.4
OM	82.4	80.3
EE	3.1	1.5
NFE	45.6	38.7
ASH	10.2	11.3

DM, dry matter; CP, crude protein; CF, crude fiber; OM, organic matter; EE, ether extract; NFE, nitrogen free extract.

Collection of data

Data collection was started after the adaptation period, and continued for five days. Before offering fresh feed in the morning, the refusal feed was weighed every day and the faecal sample was collected in polythene bags and immediately shifted to the freezer for storage. Daily feed intake, body weight gain, and the feed conversion efficiency were calculated.

Chemical analysis of feed and fecal matter

For determination of proximate analyses i.e. dry matter (DM), crude protein (CP), crude fat (CF), ether extract (EE) and nitrogen-free extract (NFE), feed and

fecal samples were air-dried and milled in Thomas Willey laboratory mill of 1 mm particle size and processed according to the method AOAC (1990).

The fecal samples obtained from each treatment were dried in an oven at 60 °C for 72 h were milled for estimation of DM digestibility, organic matter digestibility, CP, CF, NFE, EE by the method given in AOAC (1990).

In-saco *degradability (nylon bags technique)*

The *in-saco* degradability of the feed sample was performed according to the method of Orskov (1988). The feed samples were dried in the oven at 70 °C and grounded through 2 mm screen sieve in Thomas Willey Laboratory mill. The dried and clean Nylon bags having a pore size of 50µ or less and a length of 14 × 8 cm were used for the incubation of samples in the cannulated animal. About 4-5 g samples were taken in the nylon bags and tightly closed in the string approximately 24 cm long with the help of a rubber band. The bags were tightly hanged in the strings in a parallel position equally. Then the assembly was inserted into the rumen of fistulated animal. All the samples were measured in duplicate as to record accurate value. The incubation time was given for forages as 2, 4, 8, 12, 18, and 24 h. After the incubation period the bags were removed from the rumen washed with running tap water to clean the bags from feed particles contamination attached with the bags during incubation time and dried the bags at 70 °C in a hot air oven for 72 h. After drying the bags were weighted for dry matter degradability.

Statistical analysis

The effect of different feeding levels of *P. pabularia* hay on feed intake, digestibility and growth performance of sheep was calculated with the help of PROC MIXED method (Littell *et al*., 2006) of Statistical Analysis System (SAS, 2014). Statistical formula used as under: $Y_{ij} = \beta_j + \mu + \Sigma_{ij}$. Whereas: Y_{ij} = treatment yield, μ = Overall mean, β_j = effect of treatment, Σ_{ij} = Error

RESULTS

Table III shows daily nutrient intakes of Kari sheep fed alfalfa and *P. pabularia* hay. However, dry matter intake (DMI), crude protein intake (CPI), organic matter intake (OMI), ether extract intake (EEI), nitrogen-free extract intake (NFEI), ash intake (ASHI) were significantly higher ($P<0.05$) in treatment groups as compared to control. *In-vivo* nutrient digestibility of alfalfa and *P. pabularia* hay fed to Kari sheep are described in Table IV. However, dry matter

Table III. Daily nutrient intakes of Kari sheep fed alfalfa and *Prangos pabularia* hay.

Parameters (g day^{-1})	Ration A (0:70)	Ration B (24:46)	Ration C (46:24)	Ration D (70:0)	P-value
DMI	702.34^{c}±10.8	786.6^{b}±15.76	831.25^{a}±7.12	758.81^{b}±4.54	0.0002
CPI	147.21^{c} ±2.26	169.92^{b}±3.40	180.13^{a}±1.54	175.24^{ab}±1.05	0.0000
OMI	602.12^{c} ±9.24	675.98^{b}±13.4	734.16^{a}±6.29	648.63^{b}±3.88	0.0000
EEI	72.55^{c} ±1.11	92.51^{a} ±1.85	92.24^{a} ±0.89	77.36^{b} ±0.57	0.0000
Ash I	91.02^{b} ±1.39	104.08^{a}±2.08	94.43^{b} ±0.80	90.83^{b} ±0.54	0.0004
NFEI	269.71^{b} ±4.33	318.05^{a}±6.37	312.55^{a}±2.68	282.88^{b} ±1.69	0.0001

a, b, c values bearing different letters in a row differ significantly ($P<0.05$). DMI, Dry Matter Intake; OMI, Organic Matter Intake; CPI, Crude Protein Intake; EEI, Ether Extract Intake; NFEI, Nitrogen Free Extract Intake; ASHI, Ash Intake.

Table IV. *In-vivo* nutrient digestibility of alfalfa and *Prangos pabularia* hay fed to Kari sheep.

Parameters (g day^{-1})	Ration A (0:70)	Ration B (24:70)	Ration C (46:24)	Ration D (70:0)	P-value
DMD	47.53^{b}±0.95	57.94^{a}±0.75	62.42^{a} ±2.07	61.92^{a} ±1.89	0.0004
CPD	69.39^{a} ±0.43	70.08^{a} ±0.93	71.70^{a} ±1.39	70.11^{a} ±1.61	0.5894
OMD	55.39^{c} ±0.72	63.23^{b} ±0.70	67.99^{ab}±1.74	66.91^{a} ±1.63	0.0005
CFD	48.02^{b} ±0.27	58.28^{a} ±0.65	58.51^{a} ±2.31	59.42^{a} ±3.07	0.0102
Ash D	26.38^{b}±1.32	42.73^{a} ±1.16	37.98^{a}±3.49	42.99^{a} ±4.88	0.0177
NFED	58.74^{b}±1.04	66.58^{a}±0.45	66.84^{a}±1.89	68.93^{a}±1.44	0.0028
IVDMD	52.06^{c}±0.96	59.66^{b}±0.39	61.80^{ab}±0.52	60.39^{ab}±0.31	0.0000

a, b, c values bearing different letters in a row differ significantly ($P<0.05$). DMD, Dry Matter Digestibility; OMD, Organic Matter Digestibility; CPD, Crude Protein Digestibility; CFD, Crude Fat Digestibility; NFED, Nitrogen Free Extract Digestibility; AshD, Ash Digestibility.

Table V. Effect of alfalfa and *Prangos pabularia* hay on the performance parameter of Kari sheep.

Parameters	Ration A (0:70)	Ration B (24:46)	Ration C (47:24)	Ration D (70:0)	P-value
IBW (kg)	9.90^{b} ±0.15	10.90^{a} ±0.05	10.96^{a} ±0.33	10.90^{a} ±0.11	0.0001
MDI(g/day)	711.67^{d}±10.92	763.33^{b} ±6.00	799.00^{a} ±5.85	740.33^{b}±2.33	0.0001
ADG(g/day)	189.63^{a} ±3.58	200.54^{a} ±3.28	199.44^{a} ±4.22	195.90^{a}±2.80	0.0134
FCE (g/day)	3.85^{a} ±0.13	3.77^{a} ±0.07	4.00^{a} ±0.07	3.85^{a}±0.04	0.2536

a, b, c values bearing different letters in a row differ significantly (P<0.05). IBW, Initial Body Weight; MDI, Mean Daily Intake; ADG, Average Daily Gain; FCE, Feed Conversion Efficiency.

Table VI. Dry Matter and organic matter *In-saco* degradability of alfalfa and *Prangos pabularia* hay.

Rations	2h	4h	8h	12h	18h	24h
Dry matter degradability (%)						
A	59.90^{d}±0.16	61.33^{d}±0.36	68.22^{d}±0.45	78.02^{c}±0.40	79.69^{bc}±0.41	79.83^{bc}±0.45
B	61.86^{c}±0.13	66.01^{c}±0.33	70.71^{c}±0.26	77.82^{c}±0.25	78.88^{c}±0.39	78.96^{c}±0.35
C	63.98^{b}±0.73	69.06^{b}±0.28	75.53^{b}±0.35	79.71^{b}±0.28	80.87^{b}±0.33	80.71^{b}±0.09
D	67.60^{a}±0.12	72.35^{a}±0.05	79.23^{a}±0.06	86.13^{a}±0.40	87.11^{a}±0.39	87.07^{a}±0.38
P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Organic matter degradability (%)						
A	63.77^{d}±0.27	65.79^{d}±0.22	74.94^{c}±0.58	82.75^{b}±0.25	85.77^{bc}±0.19	86.05^{c}±0.45
B	65.37^{c}±0.09	68.90^{c}±0.32	73.77^{c}±0.30	80.75^{c}±0.16	85.17^{c}±0.25	86.28^{c}±0.46
C	68.17^{b}±0.60	71.55^{b}±0.47	77.66^{b}±0.34	81.66^{bc}±0.33	86.53^{b}±0.19	87.22^{b}±0.23
D	70.52^{a}±0.08	75.47^{a}±0.27	81.52^{a}±0.26	86.43^{a}±0.20	88.29^{a}±0.10	88.30^{a}±0.59
P-value	0.0000	0.0000	0.0000	0.0000	0.0028	0.0008

a, b, c values bearing different letters in a row differ significantly (P<0.05).

digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), crude fat digestibility (CFD), nitrogen-free extract digestibility (NFED), ash digestibility (AD) were significantly higher (P<0.05) in treatment groups as compared to control. The *in-vitro* dry matter digestibility for the rations A, B, C, and D were 52.06, 59.66, 61.80, 60.39%, respectively. The ration C showed the highest *in-vitro* dry matter digestibility compared to ration A, B, and D. Alfalfa and *P. pabularia* hay effect on the performance parameter of Kari sheep is shown in Table V. The initial body weight (IBW), was significantly improved in treatment groups andcompared to control, while the daily intake (MDI), average daily gain (ADG), feed conversion efficiency (FCE) were not affected. The organic matter and dry matter *in-saco* degradability at different time intervals (2h, 4h, 8h, 12h, 18h, and 24 h) were significantly affected (P<0.05) in treatment groups as compared to control (Table VI).

DISCUSSION

The *P. pabularia* is also used as a hay and winter fodder for cattle, goat and sheep in central Asia, Iran, North India, Caucasian and Pakistan district in Chitral. It is reported to be harmful in wet conditions, which probably is primarily due to the presence of some secondary metabolites in the plant (Razavi, 2012). In Chitral, during winter season the forage hay is widely used as a maintenance diet by the local farmers. The winter season for the period of six month feeding 100 kg *Prangos* hay forage met the maintenance requirements without the grain supplementation in the diet of sheep. It was observed from the study that the nutritional profile of *P. ferulacea* hay was superior forage among some common forage crops and compete with the cereal grains (Coskun, 1999; Ekinci *et al*., 2018). The current findings showed that when the level of *P. pabularia* hay in the experimental ration increased up to 50 % (ration B and C) then feed intake in terms of DMI, CPI and OMI increased

because this forage containing soluble carbohydrate.

P. ferulecea contains high digestible dry matter and high metabolizable energy (ME). The secondary metabolites such as tannins may decrease the forage intake by lowering the palatability or digestion. According to Decruyenaere *et al*. (2009) mostly the ruminants (grazing and browsing), numerous plants in temperate areas comprises of secondary metabolites like tannins, terpenes that impaired the digestibility of grazing pasture, which ultimately decreased the voluntary intake when fed to the grazing ruminants.

The nutrients digestibility of DMD, CPD, OMD, EED, AshD, and NFED were significantly higher (P<0.05) in treatment groups as compared to control. According to Yurtseven (2011) who reported that there were significant differences among *P. ferulacea*, *Astragalus gummifera* and alfalfa hay in terms of *in-vitro* gas production kinetics as well as estimated parameters such as metabolizable energy (ME) and organic matter digestibility (OMD). The ME and OMD values of *Prangos* leaves were significantly higher than those of alfalfa hay. Also, there were similar findings observed by Aldemir *et al*. (2015) who reported that all nutrients composition of *P. ferulacea* hay except crude protein was significantly greater as compared to alfalfa nutrient profile. The *in-vitro* OMD and ME values were high and the concentration of neutral detergent fiber and acid detergent fiber content were significantly lower in *P. ferulacea* hay than those of alfalfa (P<0.01). This can be suggested that *P. ferulacea* having an absolutely higher nutrients profile as that of good quality alfalfa compared to nutrient availability and digestibility rates, and thus replacement of alfalfa by *P. ferulacea* in the ration of a ruminant is practicable. Similar results were reported by Aldemir *et al*. (2015) who reported that the dry matter digestibility (DMD), *in-vitro* dry matter (IVDMD) and organic matter digestibility (IVOMD) of *P. ferulacea* hay were in competition with alfalfa hay in terms of nutritional profile.

This might be due to the less exposure of the crude protein to the body and not present in free form contains secondary metabolites. The current results are in agreement with the study of Azarfard (2008) who reported that there was no significant effect (P>0.05) among the groups of lambs for average daily DMI, ADG and FCR. However, an average daily gain of lambs fed on 100 % alfalfa hay (230 ± 21.7 g/day) was slightly higher than lambs fed on 35 %, 60 %, and 100 % of *P. ferulacea* hay. According to the Aldemir *et al*. (2015) who determined the effect of *P. ferulacea* hay after in the ration as 0 %, 20 %, 40 %, 60 %, 80 % and 100 % of total roughages diet where the rest was alfalfa feed.

The results showed that the nutrient degradability of four experimental rations was significantly affected (P<0.05) during the 18 h incubation period. According to the Aldemir *et al*. (2015) who reported that In-situ dry matter, organic matter and crude protein degradability of P. ferulacea were significantly(P<0.05) affected as compared to alfalfa hay during the first 6 h of incubation period. After the 48 h incubation period the nutrient degradability (organic matter and crude protein) were similar recorded.

CONCLUSION

It can be concluded that *P. pabularia* and Alfalfa hay is good forage based on nutrient contents and high digestibility value, so they can be used in the diet of small ruminants. There was a small biological change on average daily gain or feed conversion efficiency with the adding of *P. pabularia* and alfalfa hay in total mix ration (TMR). The *P. pabularia* and alfalfa hay as compared to other forage having high degradation kinetic and softening property can be fed to lambs for rumen enhancement.

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Compliance with ethical standards

The study was approved by the ethics committees of the Department of Animal Nutrition, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Peshawar 25130, Khyber Pakhtunkhwa, Pakistan. The ethical No. 23/2019.

Statement of conflict of interest

The authors have declared no conflicts of interest.

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