



## ***In-Vitro*, *In-Situ* and *In-Vivo* Evaluation of *Alfalfa* Residue Based Total Mixed Ration Feed Blocks for Sheep**

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### **ABSTRACT**

This study explored the potential of Alfalfa (*Medicago sativa*) residue (AR) as a protein rich concentrate for growing lambs by conducting *In-Vitro*, *In-Situ* and *In-Vivo* methods. The AR was high in crude protein (49.25%) and low in crude fiber (0.91%), with Ca: P ratio of 5.67:1.47. *In-Situ* protein degradability was very slow (RDP:UDP ratio was 10.49:89.51) and the Metabolizable energy (ME) of 4.3 MJ/kg was found. An *In-Vivo* feeding trial was conducted with 18 male lambs (3-4 months old) divided into three groups: T1 (Control) received Total Mixed Ration feed block (TMRfb) containing groundnut haulms and compounded feed mixture (50:50); T2 replaced 25% of crude protein (CP) with AR, and T3 replaced 50%. All rations were iso-nitrogenous. Results showed that DMI (% of body weight) in T2 (4.28) and T3 (4.19) had higher than T1 (4.04), but not varied significantly. Feed efficiency was similar across groups (5.41 to 5.81). Average daily gain (ADG, g/d) was higher in T2 (123.69) compared to T3 (111.29) and T1 (106.9). Nutrient digestibility and nitrogen balance were comparable among groups. Therefore, Alfalfa residue can effectively replace up to 50% of crude protein in lamb diets for its growth.

**KEYWORDS:** Alfalfa residue, *In-Situ*, *In-Vitro*, lambs, Total Mixed Ration feed block

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### **INTRODUCTION**

Feeding small ruminants in tropical regions largely depends on natural pastures and low-quality crop residues, which are often deficient in crude protein, energy and minerals. This often identified as a major factor limiting production efficiency. To address this issue, residues after the extraction of active medicinal components from legumes and other sources could serve as alternative protein sources for livestock.

Alfalfa (*Medicago sativa*), known as "the queen of forages" valued for its high biomass yield, quality forage, and palatability (Hawkins and Yu, 2018). It can be harvested after 55-80 days of sowing, producing 80-100 tons of green fodder per hectare annually under proper agronomic practices. Alfalfa contains 16-25% crude protein with 72% digestibility, 20-30% fibre, and various vitamins and minerals (Lei et al., 2017). Alfalfa forage is mechanically processed to produce chemical

components with medicinal characteristics, leaving behind a residue known as residue called as Alfalfa residue (AR) that can be used as a protein concentrate in animal feeding (Hojilla-Evangelista et al., 2017).

In view of the limited information on utilization of AR based TMR blocks in this region the present research was carried out for its nutritive evaluation by *in-vitro*, *in-situ* and *in-vivo* methods for small ruminant feeding.

### **MATERIALS AND METHODS**

#### **Procurement of ingredients and nutrient analysis**

The dry and pelleted form of Alfalfa residue (AR) was procured from a commercial firm (Kabini Enterprises, #77/4, Agasavalli, Honnali road, Kalluru, Shivamogga, Karnataka, India -577204). AR pellets (3-4mm) were stored in a clean and dry area at room temperature. About 600kg of properly

sundried groundnut haulms was procured from local area and grinded to particle size of 6-8mm using mechanical shredder. The CFM was prepared using maize, soybean meal, de-oiled rice bran, sunflower cake, molasses, mineral mixture, salt and urea at 45, 15, 10, 18, 8, 2, 1 and 1 per cent, respectively (Table 1).

All the dried samples were ground in a wiley mill (Thomas scientific, wiley® model 4 mills) to pass through 1mm screen and analyzed for proximate composition (AOAC, 2016); fiber fractions (Van Soest et al., 1991); ME and kinetics of gas production as per Rumen *in vitro* incubation and gas production (RIVIGP) of Menke and Steingass (1988). The samples were also subjected to rumen *in situ* incubation (Singh et al., 1995), the rumen degradable and undegradable fractions (Dry matter (DM) and CP) (Orskov and McDonald, 1979), the mineral content (Ca and P) was estimated by

gravimetric method as per AOAC (2005). The AR was checked for qualitative estimation for urea by spot test as per method of Garg et al. (2013).

### Preparation of TMR Feed blocks containing Alfalfa residue

The total mixed ration (TMR) feed block was formulated (Table 1) to contain around 18% CP. Premixing of CFM with molasses was made before mixing with groundnut haulms using 150kg capacity horizontal mixer for 5 minutes. The ratio of groundnut haulm and CFM was kept at ratio of 50:50 in all the treatment groups. Feed block was prepared by compressing this mix at 2800-3000 psi for 15-20 seconds using Feed Block Making Machine (HITECH HYDRAULICS, New Delhi, India. model no: DGH 04-3060-H-17) with a capacity to produce 25 to 30 blocks of 1-1.3kg per hour.

Table 1. Composition of TMR feed blocks (% as such basis) used in feeding trial

Ingredients	TMRfb-1	TMRfb-2	TMRfb-3
Groundnut haulms	50	50	50
Maize grain	22.5	23	31
Soybean mea	17.5	2.5	1
Deoiled Rice Bran	5	7	1
Sunflower cake	9	6.5	1
Molasses	4	4	4
Mineral mixture	1	1	1
3Salt	0.5	0.5	0.5
Urea	0.5	0.5	0.5
Alfalfa residue	0	5	10
Total	100	100	100

TMRfb: Total mixed ration feed block

TMRfb-1: prepared by using groundnut haulms and CFM

TMRfb-2: prepared by replacing 25% of CP of control diet with AR

TMRfb-3: prepared by replacing 50% of CP of control diet with AR

### Physical characteristics of TMR Feed Blocks

The bulk density ( $\text{kg/m}^3$ ) of the feed block was calculated by measuring the weight and its dimension in terms of length, width, and height (in cm) of the six TMRfb in each group.

### Kinetics of gas production

Air equilibrated samples ( $200 \pm 10$  mg) were incubated in 100 ml calibrated glass syringes in triplicate with 30ml mixed rumen suspension along with blanks and roughage and concentrate standards (Menke and Steingass, 1988). Cumulative gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72 and 96 hours of incubation. Data on gas production were fitted to the exponential

equation  $P = a + b(1 - e^{-ct})$ , where, P (ml) is gas production at time t; a (ml) is initial gas production; b (ml) is the potential gas production at 96 hours; and c is the rate (ml/h) of gas production (Orskov and McDonald, 1979). The metabolizable energy content and kinetics of gas production for AR, groundnut haulms and TMRfb was calculated.

### In situ incubation

The *In-Situ* technique was used to assess the protein degradability of AR in the rumen of cannulated Deoni breed bull that was used for *in-vitro* gas test. Incubation times were set at 0.1, 2, 4, 8, 16, 24, 36, 48, 72 and 96 hours. Residual DM and CP were estimated according to AOAC (2005).

The soluble or rapidly degradable fraction at 0.1h (*a*), insoluble but potentially degradable fraction (*b*), residual component at 96h of incubation (*c*), the rate of degradation (*kd*,  $1/h=0.693/t_{1/2}$ ), Potential degradability [ $Y=a+b(1-e^{-kd \times t})$ ], Effective degradability [ $P=a+b(Kd/Kd+Kp)$ ] where *Kp* is the rate of passage, taken as 0.056/h and rumen undegradable fractions (100-*P*) of AR for DM, OM and CP were obtained after fitting data to the exponential model of nonlinear regression (Orskov and McDonald, 1979).

### Feeding cum metabolic trial

Eighteen male lambs (non-descriptive types) of about 3-4 months age (an average body weight of 11.05 kg) were selected and randomly allotted into 3 groups (T1, T2 and T3) containing six each. T1: fed with TMRfb-1; prepared by using groundnut haulms and CFM; T2: fed with TMRfb-2: prepared by replacing 25% of CP of control diet with AR; T3: fed with TMRfb-3: prepared by replacing 50% of CP of control diet with AR.

The feeding trial involved two-weeks of adjustment period, followed by a twelve-week feeding trial and 5 days of metabolic trial. The lamb's diet was formulated to target a daily weight gain of 125 g, based on crude protein (CP%) and total digestible nutrients (TDN%) as per ICAR (2013) guidelines. Permission for using animals for the experiment was obtained from Institutional Animal Ethics Committee (IAEC) constituted as per Article No.13 of the CCSEA rules laid down by Government of India.

TMR feed blocks (TMRfb) were provided twice daily at 9:00 am and 4:00 pm and clean water was available throughout the day. Daily feed intake was calculated by making the difference between offered

and left feed. All the lambs were weighed every week by using digital platform balance and growth was recorded in terms of ADG (g/d). Metabolic trial was conducted at the end of feeding trial by shifting into individual metabolic cage to estimate digestibility and nutrient balance.

### Statistical analysis

Data on DMI, nutrient intake, ADG, digestibility coefficients and Nitrogen, Calcium, and Phosphorus balance were analyzed using one-way ANOVA with GraphPad Prism version 8.4.2 (GraphPad Software, La Jolla, CA, USA). Rumen *In-Vitro* gas production and *In-Situ* degradation kinetics were analyzed via non-linear regression using GraphPad Prism version 8.4.2 (GraphPad Software, La Jolla, CA, USA) to assess gas kinetics with an exponential decay equation model.

## RESULTS AND DISCUSSION

### Chemical composition of AR, groundnut haulms and experimental diets

The AR had a crude protein (CP) content of 49.25% (Table 2), consistent with findings by Madhekar and Mungikar (2009). The crude fibre (CF) content of AR was 0.91%, similar to Gawel and Grzelak (2014). The ether extract content of AR in this study was 0.41%, lower than the 1.83% reported by Homolka et al. (2012) for Alfalfa hay, due to the defatting process applied to AR production. Calcium and Phosphorus ratio of AR was 5.7:1.4. Groundnut haulms used as roughage source had more CP and compared to legume straws (Chopade et al., 2010 and Patil et al., 2024). The TMRfb were formulated to be iso-nitrogenous to evaluate AR inclusion, with CP of 18.15, 18.13 and 18.08 per cent, respectively.

Table 2. Chemical composition (% on DMB) of AR, groundnut haulms and TMRfb

Parameter	AR	GH	TMRfb-1	TMRfb-2	TMRfb-3
DM	95.8	93.5	90.8	90.9	90.9
OM	80.64	86.18	84.07	83.39	83.15
CP	49.25	16.63	18.15	18.13	18.08
CF	0.91	23.52	14.1	14.04	12.70
EE	0.41	2.56	3.960	3.50	2.74
NFE	24.66	37.3	41.84	41.89	44.17
TA	18.07	13.82	9.93	10.54	10.73
AIA	2.02	1.96	2.9	3.1	3.2
NDF	11.15	44.26	30.51	30.3	27.1
ADF	3.68	30.5	19.89	19.66	17.26
ADL	0.04	8.1	4.77	4.6	4.25
HC	7.46	13.76	10.62	10.64	9.84
C	3.64	22.4	15.12	15.05	13.01
Ca	5.67	0.75	0.48	0.75	0.97
P	1.42	0.52	0.43	0.47	0.65

### Physical characteristics of TMRfb

The Average weight (Kg) and density ( $\text{kg/m}^3$ ) of TMRfb was ranged from 1.06 to 1.10 and 686.6 to 747.9 (Table 3). Singh et al. (2016) reported a density of  $562.6 \text{ kg/m}^3$  for feed blocks prepared with wheat straw and CFM (50:50) and Samanta et al. (2003) noted that feed blocks made from natural

grass hay and CFM (60:40) had a density of 550-600  $\text{kg/m}^3$ . In the present study, the particle size of groundnut haulm and CFM was about 6-8mm and 2-3mm, respectively. Feed blocks were prepared by applying pressure of 2800-3000 psi with dwelling time of 15-20 seconds and it has produced a durable feed block of 1-1.1kg.

Table 3. Physical characteristics of TMRfb used in feeding trial

TMRfb	Weight kg	Length cm	Width cm	Height cm	Density $\text{kg/m}^3$
TMRfb-1	1.06	16.2	15.4	6.2	686.6 (19.44 $\text{kg/ft}^3$ )
TMRfb-2	1.10	15.7	15.3	6.1	747.9 (21.18 $\text{kg/ft}^3$ )
TMRfb-3	1.06	15.9	15.3	6.2	701.5 (19.86 $\text{kg/ft}^3$ )

### Rumen *In vitro* incubation for gas production (RIVIGP)

The RIVIGP values (RIVIGP<sup>24</sup>, *a*, *D*, *k*, *t*<sub>1/2</sub> and RIVIGP<sup>96</sup>) of AR, groundnut haulm and TMRfb used in the feeding trial are presented in Table 4. Babiker et al. (2017) reported ME value of 7.1 MJ/kg DM in Alfalfa hay. The rate and extent of gas production in AR was lower in present study. The lower ME (4.3 MJ/kgDM) and RIVIGP<sup>24</sup> ml/200mg DM (15.17) in AR was may be due to processing methods used in its production. By application of

pressure and maceration about 55–60% of the juice from fresh plant will be removed (Arlabosse et al., 2011) and along with this application of temperature (Koschuh et al., 2004 and Damborg et al., 2020) alters plant structure and impaired the accessibility of nutrients to rumen microbes during *in vitro* analysis for its gas production for ME estimation. Inclusion of AR in TMRfb2 and TMRfb3 reduced gas production (RIVIGP<sup>24</sup> ml/200mg DM) and ME (MJ/kgDM) by 7 and 17 per cent and 6.86 and 13.73 per cent, respectively in comparison with TMRfb1.

Table 4: Kinetics of gas production of AR, GH and feed blocks

Feed sample	ME (MJ/kg DM)	RIVIGP <sup>24</sup> ml/200mg DM	RIVIGP <sup>96</sup> ml/200mg DM	<i>a</i>	<i>D</i>	<i>k</i>	<i>t</i> <sub>1/2</sub>
AR	4.3	15.17	24.87	1.39	26.21	0.04	19.62
GH	8.0	37.78	47.69	0.62	45.99	0.09	7.69
TMRfb1	9.10	49.07	56.69	6.56	52.28	0.08	9.56
TMRfb2	8.75	46.96	56.26	2.93	51.61	0.06	10.38
TMRfb3	8.40	44.30	50.40	1.96	47.35	0.061	0.62

RIVIGP<sup>24</sup>- Rumen *in vitro* Gas Production at 24 hours; RIVIGP<sup>96</sup>- Rumen *in vitro* Gas Production at 96 hours; *a*-Rapidly produced gas (ml/200mg DM); *D*- potential gas production (ml/200mg DM); *K* ( $\text{h}^{-1}$ ) – Rate of production per hour; *t*<sub>1/2</sub> – time at which half of the gas is produced

### Kinetics of degradability

The protein degradability of AR was determined as per Orskov and McDonald (1979) method of *in-situ* degradability (Table 5). The degradability percent for DM, OM and CP were 25.57, 15.97, 58.46, 0.000001, 41.54 and 25.57; 17.50, 21.87, 60.63, 0.0012, 39.37 and 18.34; and 9.82, 27.17, 63.01, 0.001, 36.99 and 10.49, respectively.

Lu and Jorgensen (1997) reported 35.3 per cent protein degradability for AR. In this study the ratio

of RDP:UDP of AR was 10:90. The lower protein degradability of AR was due to processing methods applied during its production (Lu et al., 1988, Koschuh et al., 2004 and Damborg et al., 2020). The ADIN of AR was only about 1.21 per cent (or 7.56 per cent of total CP), indicating that its UDP (90% of CP) is further digested enzymatically and not lost through dung.

Table 5. *In situ* rumen degradability of DM, OM and CP (per cent) of AR

Variables	DM	OM	CP
<i>a</i>	25.57	17.50	9.82
<i>b</i>	15.97	21.87	27.17
Degradability at 24h	29.67	23.52	17.34
<i>c</i>	58.46	60.63	63.01
$k_d$ ( $h^{-1}$ )	0.000001	0.001215	0.001034
<i>Y</i>	41.54	39.37	36.99
$P^1$	25.57	18.34	10.49 (RDP)
Rumen undegradable Dm <sup>2</sup>	74.43	81.66	89.51 (UDP)
ADIN (%)	1.21		

*a* = soluble or rapidly degradable fraction; *b* = insoluble but potentially degradable fraction; *c*=Residual dry matter at 96 h of incubation;  $k_d=0.693/t_{1/2}$ , degradation rate ( $h^{-1}$ ); [*a*, *b* and  $k_d$  are based on nonlinear regression using the exponential model]; *Y* is fraction degradation at time *t*,  $Y=a+b(1-e^{-k_d t})$ ; <sup>1</sup>effective degradability,  $P=a+b(K_d/K_d+K_p)$  [ $K_p$  is the rate of passage, taken as 0.056/h]; <sup>2</sup>(100-*P*).

### Average nutrient intake and nutrient density

The average daily dry matter intake (DMI) of TMR feed blocks ranged from 614.20 g/day for T1 to 666.70 g/day for T2 and DMI (% BW) from 4.04 (T1) to 4.28 per cent (T2) but difference was non-significant ( $P \geq 0.05$ ).

Nutritive value of diet in terms of DCP (%), TDN (%) and ME (MJ/kg), along with intake of DCP (g/d), TDN (g/d), and ME (MJ/d), are given in Table 6. There were no significant differences in CP, DCP and TDN intake across groups. The average crude protein intake (CPI) was 111.48 g/d, T1; 120.87 for T2; and 114.84 g/d, for T3. CPI differences were also non-significant ( $P \geq 0.05$ ) and the all lambs were

provided sufficient protein (109.09 g/d) to achieve ADG of 125g/d as stipulated by ICAR (2013) feeding standard. In the entire feeding trial, an additional intake of about 2-5g of CP was found. Whereas, the intake of DCP and energy was lower than the ICAR (2013) standard requirements for an ADG of 125 g/day. Pavan et al. (2024) reported a lower DMI of 3.64% for sorghum-based TMRfb, likely due to its higher roughage portion (60:40) and NDF intake. Rani et al. (2016) found no significant difference in dry matter intake when replacing soybean meal with Alfalfa residue pellets at 20% inclusion in calf rations, indicating similar palatability to conventional protein sources.

Table 6. Average intake of nutrients in lambs during feeding trial

Parameter	T1	T2	T3	SEM	P
Average body weight (kg)	15.81	16.15	15.78	0.33	0.81
DMI (g/d)	614.20	666.70	635.18	18.14	0.53
% body weight	4.04	4.28	4.19	0.08	0.54
CP (%)	18.15	18.13	18.08	0.01	0.001
DCP (%)	9.43	10.01	9.60	0.30	0.75
TDN (%)	60.47	58.69	56.92	0.75	0.17
ME (MJ/kg) <sup>#</sup>	9.10	8.75	8.40	-	-
CP intake (g/d)	111.48 (109.09)	120.87 (109.98)	114.84 (109.01)	3.29	0.53
DCP intake (g/d)	57.91 (73.10)	66.71 (73.67)	60.95 (73.05)	2.50	0.36
TDN intake (g/d)	371.41 (431.13)	391.29 (435.63)	361.54 (430.73)	8.88	0.37
ME intake (MJ/d) <sup>#</sup>	5.58 (6.52)	5.83 (6.59)	5.33 (6.52)	0.20	0.51

$P < 0.01$ ,  $P \leq 0.05$ , Means bearing different superscripts between the columns differ significantly and  $P \geq 0.05$  is non-significant. Values in parenthesis are the requirements as stipulated by the ICAR (2013) for the average body weight of lambs with ADG of 125 g/d.

### Digestion cum metabolic trial: Nutrient digestibility

The intake of NDF and ADF (Table 7) in all the groups was adequate and at levels required for optimum fermentation in rumen (Van Soest et al., 1991; NASEM, 2021).

The digestibility of DM, OM, CP, CF, NDF and ADF between the treatment groups had no significant difference and the values ranged from 63.81 (T3) to 69.23 (T1); 70.27 (T1) to 71.89(T2);

51.95 (T1) to 55.19 (T2); 50.05 (T3) to 54.21 (T2); 59.36 (T3) to 62.93 (T2); and 52.79 (T3) to 59.96 (T1) respectively. No significant difference ( $P \geq 0.05$ ) for nutrient digestibility was observed between the treatment groups. Nutrient digestibility between the treatment group showed no significant difference in a study conducted by Rani et al. (2016) where soybean meal was replaced Alfalfa residue pellets at 20% level. The digestibility values of CF, EE, NDF, and ADF were similar to the values reported in the present study.

Table 7. Mean intake of nutrients and digestibility (%) during metabolism trial

Parameter		T1	T2	T3	SEM	P-value
DM	g/d	823.37	891.18	848.67	27.91	0.68
	% Body weight	4.06	4.20	4.14	0.14	0.82
	Digestibility	69.23	66.00	63.81	0.99	0.08
OM	g/d	669.54	739.66	697.71	22.77	0.62
	% Body weight	3.36	3.46	3.41	0.08	0.84
	Digestibility	70.27	71.89	70.33	1.67	0.54
CP	g/d	137.87	159.71	144.24	6.05	0.47
	% Body weight	0.70	0.75	0.71	0.02	0.53
	Digestibility	51.95	55.19	53.07	1.18	0.75
CF	g/d	113.85	126.48	107.24	4.35	0.19
	% Body weight	0.58	0.59	0.52	0.02	0.28
	Digestibility	53.09	54.21	50.05	1.16	0.32
NDF	g/d	237.84	260.70	223.00	9.18	0.27
	% Body weight	1.20	1.22	1.09	0.03	0.24
	Digestibility	62.60	62.93	59.36	1.76	0.27
ADF	g/d	151.98	155.16	136.53	5.93	0.41
	% Body weight	0.76	0.73	0.67	0.03	0.49
	Digestibility	59.96	55.52	52.79	1.77	0.68

$P < 0.01$ ,  $P \leq 0.05$ , Means bearing different superscripts between the columns differ significantly and  $P \geq 0.05$  is non-significant.

### Growth performance, feed efficiency and balance of nutrients

The total body weight gain (kg) for 12 weeks of feeding trial was 8.59, 10.39 and 9.35 for T1, T2 and T3, respectively (Table 8). The respective average daily gain (ADG, g/d) was 106.90, 123.69 and 111.29. Feed efficiency was not significantly different ( $P \geq 0.05$ ) among the treatment groups. T2 had the best feed efficiency (5.41), followed by T3 (5.71) and T1 (5.81).

The diet for the experimental lambs was formulated to target a daily gain of 125 g (ICAR, 2013) based on CP (%) and TDN (%). While the T2 group achieved this target, the ADG in T1 and T3 was 14 to 19 g lower, though the difference was non-significant, indicating uniform nutrient intake across all groups using TMRfb. Pavan et al. (2024)

reported ADG of 85.2 g/d for sorghum-based TMRfb fed lambs. Whereas, in this study, the quality of TMRfb was much superior (TDN, DCP and digestibility) hence higher gain and efficiency was observed. In the present experiment, the positive nitrogen balance was observed in all the treatment which is suggesting nutritional adequacy with respect to energy and protein supply for maintenance and growth. The higher nitrogen retention in T2 was attributed to greater crude protein digestibility compared to the other groups. There was a significant difference ( $P < 0.001$ ) in calcium intake more in T3 and T2 due to presence of AR in these TMRfb (Table 2). Although higher retention in T3 and T2, but of non-significant. Similar trend was noticed with regard to phosphorus metabolism (Table 8).

Table 8. Growth performance, feed efficiency and balance of nutrients during the feeding trial

Parameter	T1	T2	T3	SEM	P
Initial body wt. (kg)	11.32	10.96	11.11	0.23	0.83
Final body wt. (kg)	20.30	21.35	20.46	0.53	0.71
Total weight gain (kg)	8.98	10.39	9.35	0.57	0.61
ADG (g)	106.90	123.69	111.29	6.81	0.61
Feed efficiency	5.81	5.41	5.71	0.36	0.57
Nitrogen balance					
Nitrogen intake (g)	22.99	25.72	23.38	1.93	0.49
Nitrogen outgo (dung + urine) (g)	9.94	8.96	8.88	0.66	0.17
Absorbed nitrogen (g)	18.10	21.99	19.90	0.94	0.38
Retained nitrogen (g)	13.05	16.76	14.49	0.92	0.28
N retained/ N intake (%)	55.84	64.94	60.95	4.63	0.09
N retained/ N absorbed (%)	69.79	76.08	71.73	1.55	0.17
Calcium balance					
Calcium intake (g)	3.92 <sup>c</sup>	6.66 <sup>b</sup>	8.29 <sup>a</sup>	0.48	0.001
Calcium excretion in dung	1.75 <sup>c</sup>	2.75 <sup>b</sup>	3.71 <sup>a</sup>	0.22	0.001
Calcium excretion in urine	0.54 <sup>b</sup>	0.70 <sup>ab</sup>	0.81 <sup>a</sup>	0.05	0.04
Ca retained/ Ca intake (%)	40.63	47.93	44.53	2.40	0.51
Phosphorus balance					
Phosphorus intake (g)	3.34 <sup>b</sup>	4.01 <sup>b</sup>	5.44 <sup>a</sup>	0.28	0.001
Phosphorus excretion in dung	1.42 <sup>b</sup>	1.85 <sup>ab</sup>	2.12 <sup>a</sup>	0.10	0.01
Phosphorus excretion in urine	0.24	0.20	0.20	0.01	0.41
P retained/ P intake (%)	49.72	48.72	56.33	1.77	0.13

P<0.01, P≤0.05, Means bearing different superscripts between the columns differ significantly and P≥0.05 is non-significant

## CONCLUSION

The AR is found to have higher per cent of CP (49.25) and Ca:P ratio (5.7:1.4) which would be one of the better sources of nutrient for the small ruminants. However, the rumen degradability found to be lower indicating good bypass protein value because of processing methods during extraction of compounds from Alfalfa forage. Replacement of AR up to 50 per cent of protein requirement did not affect the intake of nutrient, gain and digestibility in small ruminants.

## REFERENCES

- AOAC. 2016. Official methods of analysis of the Association of Official Analytical Chemists, 20th Edn., AOAC Inc., Washington DC.
- Arlabosse, P. and Blanc, M., 2011, October. Thermo-mechanical fractionation of green biomass: optimisation of the processing conditions according to the water potential of the tissue. In European Drying Conference. pp.26-28.
- Babiker, E.E., Juhaimi, F.A., Ghafoor, K. and Abdoun, K.A., 2017. Comparative study on feeding value of Moringa leaves as a partial replacement for Alfalfa hay in ewes and goats. *Livestock Science*. 195: 21-26.
- Chopade, S., Kalbande, V.H., Shelke, S.K. and Dandage, S.D. 2010. Growth performance and economics of urea treated soybean straw based pelleted complete ration in kids. *Indian Journal of Animal Nutrition*. 27: 138-141.
- Damborg, V.K., Jensen, S.K., Weisbjerg, M.R., Adamsen, A.P. and Stodkilde, L. 2020. Screw-pressed fractions from green forages as animal feed: Chemical composition and mass balances. *Animal Feed Science and Technology*. 261: 114401.
- Garg, M.R., Sherasia, P.L. and Bhandari, B.M., 2013. Quality control manual for cattle feed plants. Animal Nutrition Group, National Dairy Development Board, Anand-388. 1: 8.

- Gaweł, E. and Grzelak, M. 2014. Protein from Alfalfa in animals supplement diet. *Journal of Food, Agriculture and Environment*. 12 (2): 314-319.
- Hawkins, C. and Yu, L.X. 2018. Recent progress in Alfalfa (*Medicago Sativa L.*) genomics and genomic selection. *Crop Journal*. 6(6): 565-575.
- Hojilla-Evangelista, M.P., Selling, G.W., Hatfield, R. and Digman, M. 2017. Extraction, composition, and functional properties of dried AR (*Medicago sativa L.*) leaf protein. *Journal of the Science of Food and Agriculture*. 97(3): 882-888.
- Homolka, P., Koukolova, V., Podsednicek, M. and Hlavackova, A. 2012. Nutritive value of red clover and lucerne forages for ruminants estimated by in vitro and in vivo digestibility methods. *Czech Journal of Animal Science*. 57 (10): 454-568.
- ICAR. 2013. Nutritional Requirements of Sheep, Goat and Rabbit, 2nd Edn., Indian Council of Agricultural Research, New Delhi, pp.7-8.
- Koschuh, W., Povoden, G., Thang, V.H., Kromus, S., Kulbe, K.D., Novalin, S. and Krotscheck, C. 2004. Production of leaf protein concentrate from ryegrass (*Lolium perenne x multiflorum*) and Alfalfa (*Medicago Sativa*) Comparison between heat coagulation/centrifugation and ultrafiltration. *Desalination*. 163 (1-3): 253-259.
- Lei, Y., Hannoufa, A. and Yu, P. 2017. The use of gene modification and advanced molecular structure analyses towards improving Alfalfa forage. *International Journal of Molecular Science*. 18 (2): 298.
- Lu, C.D. and Jorgensen, N.A. 1997. LNR Protein Concentrate as a Less Ruminally Degradable Protein Source for High-Producing Dairy Cows. *Journal of Agricultural and Marine Sciences*. 2:31-35.
- Lu, C.D., Jorgensen, N.A. and Satter, L.D., 1988. Site and extent of nutrient digestion in lactating dairy cows fed LNR protein concentrate or soybean meal. *Journal of Dairy Science*. 71 (3):697-704.
- Madhekar, R.D. and Mungikar, A.M. 2009. Chemical composition of Alfalfa (*Medicago sativa L.*) leaf meal and leaf protein concentrate. *Journal of Phytological Research*. 22: 95-98.
- Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal Research and Development*. 28: 7-55
- NASEM, 2021. National Academies of Sciences, Engineering and Medicine, 2021. Nutrient Requirements of Dairy Cattle, 8th Edn. National Academy Press, Washington, DC, The National Academies Press.
- Orskov, E.R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science*. 92 (2): 499-503.
- Patil, P.V., Gendley, M.K. Dubey, M. Dhok, A.P. and Prusti. S. 2024. Effect of Feeding Gram Straw based Complete Feed Pellets on Rumen Fermentation and Haemato-biochemical Parameters in Goats. *Indian Journal of Animal Nutrition*. 41 (2): 280-287.
- Pavan B.L., V. Nagabhushana, G.U. Manju, K.S. Giridhar, N. Suma and B.U. Umesh. Effect of Feeding Sorghum Hay, Maize Stover and Areca Sheath Based Total Mixed Ration Feed Block (TMRfb) on Nutrient Intake, Digestibility and Cost Economics in Growing Lambs. *Indian Journal of Animal Nutrition*. 2024. 42 (1): 54-60.
- Rani, K.J., Ally, K., Seethal, C.C., Surej, J.B. and Rajkumar, G. 2016. Effect of dietary incorporation of nutraceutical residue on dry matter intake and nutrient digestibility in crossbred calves. *Journal of Veterinary and Animal Science*. 47 (2): 78-81.
- Samanta, A.K., Singh, K.K., Das, M.M., Maity, S.B. and Kundu, S.S. 2003. Effect of complete feed block on nutrient utilisation and rumen fermentation in

- Barbari goats. *Small Ruminant Research*. 48 (2): 95-102.
- Singh, C.K., Robinson P.H. and Mc Niven, M.A. 1995. Evaluation of raw and roasted lupin seeds as protein supplement for lactating cows. *Animal Feed Science and Technology*. 52: 63-76.
- Singh, P.K., Chandramoni, Kumar, K. and Kumar, S. 2016. Effect of feeding wheat and rice straw based complete feed blocks on nutrients utilization, blood biochemical and growth performance in crossbred calves. *Indian Journal of Animal Science*. 86 (7): 771-776.
- Van Soest, P.V., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 74 (10): 3583-3597.