



Intake, nutrient metabolism and balance studies in sheep fed Himalayan Elm (*Ulmus wallichiana*) leaf meal incorporated total mixed ration

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ABSTRACT

With the soaring cost and scarcity of concentrate feeds worldwide, use of un-conventional tree foliages in the animal diets can be effective in curtailing the feeding costs to economise livestock production. The present experiment was conducted with the aim to assess incorporation of Himalayan Elm (*Ulmus wallichiana*) leaf meal (ELM) as partial replacement to conventional concentrate feed mixture (CFM) in total mixed ration (TMR) of sheep. In Experiment-I (*in vitro* study), the effect of graded increasing levels (0, 10, 20, 30, 40 and 50%) of ELM inclusion as replacement to CFM constituting 40% in the basal TMR on nutrient degradability was assessed. The results revealed the effective level for optimal nutrient degradability at 30% ELM inclusion. In Experiment-II (*in vivo* study), a feeding trial was conducted for 30 days on sheep (16) distributed equally into two groups, offered basal TMR as control (Cont) and TMR containing ELM replacing CFM at 30% level as treatment group (ELMG), the level selected based on results of optimum nutrient degradability in *in vitro* assay. Without affecting dry matter and organic matter intakes, ELM included diet enhanced crude protein and neutral detergent fibre digestibility. Moreover, content and the intakes of digestible nutrients were similar between the two groups, with total digestible nutrients intake being 56.66% and 61.88% more than the recommendation in control and ELMG groups, respectively. Animals of both the groups were in positive balances of N, Ca and P, though N balances (g/d) and live weight gain (g/d) were comparable while Ca and P balances (g/d) were higher in ELMG. Cost of production lowered with 35.84% reduction in feed cost per kg live weight gain in ELMG group compared to control. It was concluded that 30% CFM in TMR for sheep could be replaced with ELM safely as a sustainable strategy to alleviate the conventional feed shortage for profitable sheep rearing.

Keywords: Balances, Economics, Elm foliage, Nutrient utilization, Replacement

Tree foliages are generally considered an emergency fodder for livestock, but form an integral part of ruminant feeding system in the hilly zones all over the world (Bakshi and Wadhwa 2007). These feed resources make significant contribution to meet the nutritional requirements of ruminant livestock by mitigating some of the feed shortages and deficiencies experienced especially during lean season (Parissi *et al.* 2018), and thus have been successfully incorporated in the diets of small ruminants (Beigh *et al.* 2020). Fodder tree leaves are used as a cheaper source of supplements to the quantity and quality of pastures for grazing livestock, and even can replace conventional concentrates.

The Jammu and Kashmir is bestowed with plenty of top fodder foliages, among which Himalayan Elm (*Ulmus*

wallichiana) is one of the nutritious foliages. The tree, also known as Kashmiri Elm is a deciduous mountain tree ranging from Central Nuristan in Afghanistan, through Northern Pakistan and Kashmir in Northern India to Western Nepal at elevations from 800 to 3,000 m (Batoool *et al.* 2014). The Elm foliage is preferred over most other tree species for use as fodder for ruminant livestock, and has been fed as sole diet to goats for intensive animal feeding in our previous study (Beigh *et al.* 2020). However, its optimal inclusion level as replacement to costly conventional concentrate feeds has not yet been determined. In this context with the aim to economise ruminant feeding by reducing dependence on conventional concentrate feeds, the present study was conducted to *in vitro* scrutinize the optimum level of Elm leaf meal (ELM) inclusion in total mixed ration (TMR) of sheep and to assess *in vivo* effect of partial replacement of concentrate feed mixture (CFM) with the ELM in TMR on feed intake, nutrient utilisation, nutritional profile, mineral balances, growth performance and production economics of sheep.

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MATERIALS AND METHODS

Collection of tree foliage: Foliage of *U. wallichiana* were collected at the cultivated nursery site of Faculty of Forestry, SKUAST-Kashmir, Shuhama located at 34°5′–34°7′ N latitude, 74°8′–74°9′ E longitude in late summer months (August and September) when the mean atmospheric temperature ranges between 25 to 35°C and relative humidity varies from 59 to 68%. Around 25 trees (approximately 5 years old) were identified and harvested for foliage which was pooled and a sub-sample was considered for *in vitro* assay. For *in vivo* study, tree branches were lopped, leaves were trimmed, dried, milled to prepare the leaf meal, and then stored until the trial.

***In vitro* study:** The *in vitro* study was carried out to determine the response of CFM replacement by ELM in TMR at graded levels on nutrient degradability to select the effective level that can be used for animal feeding. For this purpose, six oats straw based TMR's with 60 : 40 roughage to concentrate ratio were formulated in which the ELM was incorporated replacing CFM at 0 (L_0), 10 (L_1), 20 (L_2), 30 (L_3), 40 (L_4) and 50% (L_5) levels, respectively where L_0 served as control. The CFM comprised of crushed maize, wheat bran, cottonseed cake, soybean, urea and molasses, the mixtures being fortified with vitamin-mineral premix and common salt.

Rumen fluid was collected from three adult donor Corriedale sheep fed *ad lib.* on a diet of oats straw and commercial concentrate feed in a ratio of 60 : 40 with free access to fresh water. About 250 mL of rumen fluid was collected from each animal in early morning before feeding through a stomach tube. The *in vitro* procedure as per Tilley and Terry (1963) method was followed to determine the nutrient degradability at 48 h post incubation. *In vitro* nutrient (i.e. dry matter, organic matter and neutral detergent fibre) degradability was calculated as the nutrient which disappeared from the initial weights inserted into the flasks.

***In vivo* study:** Sixteen male Corriedale sheep (23.43±0.47 kg body weight) of uniform age (13–14 months) and body conformation were distributed into two equal groups. Animals of one group designated as control (Con) were fed basal TMR containing oats straw 60 parts and CFM 40 parts, while animals of the other group designated as treatment (ELMG) were fed the TMR based on 60 parts of oats straw and 40 parts of CFM replaced (on weight basis) by ELM at 30% level (i.e. containing 28 parts of CFM and 12 parts ELM), so chosen on basis of the best results of *in vitro* nutrient degradability assay.

The feeding experiment was conducted for a period of 30 days during which weighed quantities of feed were offered to animal individually twice daily for the entire experimental periods to meet their nutrient requirement for the body weight gain of 50–100 g/day as per recommendations of Kears (1982). Refusal feed was quantified daily to determine feed intake. Clean and fresh drinking water was offered *ad lib.* twice daily. The body weights (BW) of experimental animals were recorded at

weekly intervals in the morning before feeding and watering.

Towards the end of feeding trial, a metabolism trial was carried out during which five representative animals from each group were kept in metabolism cages well equipped with drinking and feeding facilities besides collection of feces and urine separately. The metabolism trial consisted of 3 days of adaptation followed by 6 days for collection period during which records of feed intake, orts, faeces and urine voided were maintained, and representative samples (10% of daily production) were taken daily, pooled for each animal for the collection period and sub-sampled for analysis. Urine samples were collected in plastic containers, containing 20 mL of concentrated sulfuric acids to prevent ammonia losses, and stored at –20°C until chemical analysis. The DM of feed and feces was determined by drying in a forced air oven at 60°C for 48 h. The dried samples were ground using a Wiley mill to pass a 1 mm screen before chemical analyses.

Upon completion of the experimental trials, economics of feeding was calculated by taking into consideration the market prices of feed ingredients at the time of purchase, cost of processing (labour cost, electricity charges, repair and maintenances, etc.), feed consumed and body weight gain of animals.

Sample analysis: The non-fermented residues left after 48 h of incubation in *in vitro* assay were analysed for dry matter (DM), organic matter (OM) and neutral detergent fibre (NDF). Feed samples of *in vitro* and *in vivo* assays were analysed for proximate composition (AOAC 2000). Feed and faecal samples were also analysed for NDF and acid detergent fibre (ADF) as per Van Soest *et al.* (1991) and macro-mineral contents, viz. calcium (Ca) and phosphorus (P) as per Talapatra *et al.* (1948). Urine was analysed for nitrogen, Ca and P. The nutrient digestibility were measured based on the amount of nutrient consumed and excreted. Digestible nutrient contents of the diets were calculated from the chemical composition and average apparent *in vivo* total tract nutrient digestibility coefficients. The digestible energy (DE Mcal/kg) was determined from apparent digestible nutrients by using the Rostock equation (Schiemann *et al.* 1971), which was converted into metabolisable energy (ME Mcal/kg) by multiplying with the factor 0.82.

Statistical analysis: The *in vitro* experiment was completed in one run, using single ruminal inoculum for all the replacement levels of CFM with ELM. The contrast was designed to test linear response of the degradability parameters to the gradual increasing replacement levels. While, the data of *in vivo* assay was subjected to test of significance (Independent-samples t-test) using statistical software program SPSS (version 20.0) for Windows. Any P value less than 0.05 ($P < 0.05$) was taken to be statistically significant.

RESULTS AND DISCUSSION

Chemical composition of diets: The chemical

Table 1. Chemical composition of TMR's with concentrate feed mixture replaced with Elm leaf meal at graded levels

Attribute	Levels of Elm leaf meal [#]					
	L ₀	L ₁	L ₂	L ₃	L ₄	L ₅
Organic matter	92.70	91.85	91.10	91.00	91.00	90.95
Crude protein	13.67	13.12	12.81	12.68	12.22	12.16
Ether extract	7.90	6.90	6.62	6.50	6.43	6.34
Non-structural carbohydrate	40.13	40.22	40.98	41.21	41.35	41.69
Neutral detergent fibre	65.90	66.70	66.90	67.90	72.50	73.57
Acid detergent fibre	38.18	39.32	40.07	40.27	43.74	43.90
Calcium	1.37	1.43	1.45	1.52	1.55	1.56
Phosphorus	0.81	0.86	0.85	0.83	0.84	0.82

[#]Levels of concentrate feed mixture replacement with Elm leaf meal in TMR's: L₀, Control (basal TMR without replacement); L₁, TMR with 10% (w/w) replacement of CFM; L₂, TMR with 20% (w/w) replacement of CFM; L₃, TMR with 30% (w/w) replacement of CFM; L₄, TMR with 40% (w/w) replacement of CFM; L₅, TMR with 50% (w/w) replacement of CFM.

composition of the basal (control) and five experimental TMR's is presented in Table 1. Crude protein (CP) contents were 26.54, 14.21 and 4.80%, ether extract (EE) were 9.10, 8.70 and 1.03%, while NDF contents were 31.78, 46.15 and 70.31% in CFM, ELM and oats straw, respectively. Lower CP and EE, while higher fibre fractions contents in ELM than CFM resulted in gradual decrease in CP, EE and OM while increase in fibre contents with the increase of replacement levels. The chemical composition of Elm foliage reported in the present investigation is comparable to our earlier study (Beigh *et al.* 2020).

In vitro nutrient degradability: Partial replacement of CFM by graded levels of ELM in TMR increased *in vitro* degradability of DM, OM and NDF. A higher ($P < 0.05$) nutrient degradability was recorded with the replacement of CFM by ELM at 30% level (Fig. 1). The *in vitro* nutrient degradability improved with ELM inclusion in TMR's which could be attributed to alleviation of mineral deficiencies (Hove *et al.* 2001) and rumen unfavourable conditions which predispose due to supplementation of high concentrate diet (Olafadehan *et al.* 2020), thereby modify rumen environment towards higher intensity of rumen microbial activity, and thus higher nutrient degradability. However, replacing CFM with ELM at higher levels (>30%) tended to reduce *in vitro* DM and NDF degradability in L₄ and L₅ compared to L₀ probably due to increase in non-structural carbohydrates and decrease in crude protein contents in the respective levels. Faster ruminal degradation of the relatively higher NSC, a readily fermentable carbohydrate fraction, may lower ruminal pH beyond threshold, cause acidosis that adversely affects nutrient digestion (Olafadehan *et al.* 2020).

In vivo voluntary intakes: The intakes (g/d and g/kg W^{0.75}) of DM, OM and digestible nutrients were comparable

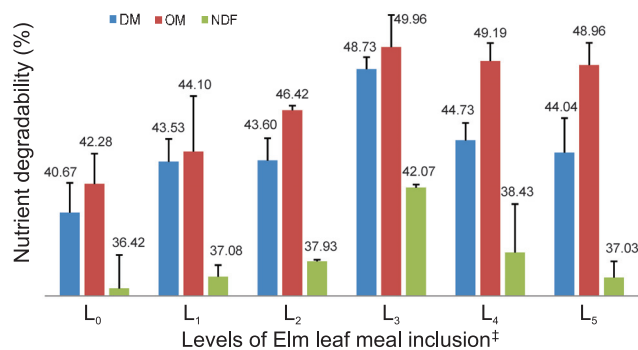


Fig. 1. *In vitro* nutrient degradability (% DM) of TMR's incorporated with gradual increasing levels of Elm leaf meal at 48 h of incubation. [‡]Levels of conventional CFM substituted with ELM in TMR's: L₀, Control (basal TMR without substitution); L₁, TMR with 10% (w/w) substitution of CFM; L₂, TMR with 20% (w/w) substitution of CFM; L₃, TMR with 30% (w/w) substitution of CFM; L₄, TMR with 40% (w/w) substitution of CFM; L₅, TMR with 50% (w/w) substitution of CFM; DM, dry matter, NDF, neutral detergent fibre, OM, organic matter.

between the two dietary treatments (Table 2), though numerically higher in treatment group. Inclusion of ELM for replacement of CFM at 30% level in the TMR does not affect the intakes as compared to those fed with the TMR without the leaf meal, indicating that both the experimental diets were palatable. Similar trend observed for intakes of digestible nutrients could be due to the similarity in DM intakes, as intake of nutrients is a function of nutrient content and DM intake. TDN intake in control and treatment groups was 56.66 and 61.88% more than the recommended requirements (Kearl 1982) to support 100 g daily weigh gain in sheep. The results of this study confirmed the findings of Ganai and Beigh (2015) who reported that

Table 2. Voluntary intakes in sheep fed TMR with partially replaced concentrate feed mixture by Elm leaf meal

Attribute		Treatment groups [‡]		P value
		Con	ELMG	
DM	g/d	909.95±10.81	925.52±8.81	0.283
	g/kg W ^{0.75}	83.91±0.88	85.28±0.60	0.219
OM	g/d	843.52±10.02	842.22±8.02	0.921
	g/kg W ^{0.75}	77.78±0.81	77.61±0.55	0.860
Digestible DM	g/day	547.29±13.63	583.08±15.48	0.105
	g/kg W ^{0.75}	49.94±1.09	53.00±1.05	0.062
Digestible OM	g/day	567.38±12.68	586.48±13.63	0.322
	g/kg W ^{0.75}	51.77±0.94	53.31±0.83	0.240
Digestible CP	g/day	78.80±1.87	79.14±1.92	0.902
	g/kg W ^{0.75}	7.19±0.14	7.19±0.12	0.570
TDN	g/day	626.64±14.06	647.53±15.02	0.327
	g/kg W ^{0.75}	57.19±1.07	58.87±0.91	0.254

[‡]Treatment groups: Cont, Control (animals fed control diet, i.e. basal TMR without replacement); ELMG, animals fed TMR with 30% (w/w) CFM replaced with ELM.

complete diet based on leaf meal mixture as replacement to concentrate mixture at 30% level could be fed to sheep without any adverse effect on feed intake. Similarly, Das *et al.* (2012) reported that there were no significant differences in voluntary feed intakes in crossbred heifers fed diets with replaced concentrate mixture protein by stylo meal.

In vivo nutrient digestibility and nutritive value of diets: Digestibility of CP and NDF were higher ($P<0.05$) in ELMG, while the digestibility of all other nutrients were not different between the two groups; however, nutrient density (% DCP, % TDN, NR, DE and ME) of diets of both control and treatment groups were comparable (Table 3). The ELM added group had higher CP digestibility probably due to positive influence of the leaf meal on rumen environment in animals due to the presence of low level of tannins (15.67 g/kg DM) in the Elm foliage because of agro-climatic and edaphic conditions of the region as reported in our previous study (Beigh *et al.* 2020). Moderate level of tannins (<40 g/kg DM) in forages can have advantage in ruminants by acting as organic protectants of dietary proteins from rumen microbial degradation (Makkar 2003). Increased fibre (NDF) digestibility of ELMG diet compared to control diet might be due to decrease in the passage rate and increase of the mean retention time of feed in the rumen due to the bulkiness of foliage. Similarly, Das *et al.* (2012) also observed improvement in fibre digestibility in crossbred heifers fed diet with CFM replaced by stylo meal either at 50% or 100%, level.

Table 3. Nutrient digestibility and nutritive value of TMR with partially replaced concentrate feed mixture by Elm leaf meal

Attribute	Treatment groups [‡]		<i>P</i> value
	Con	ELMG	
<i>Nutrient digestibility</i>			
Dry matter	60.01±1.04	62.03±1.12	0.210
Organic matter	67.11±0.87	68.56±0.92	0.272
Crude protein	63.21±0.97	66.39±0.99	0.038
Ether extract	64.02±0.89	64.64±1.04	0.658
Non-structural carbohydrates	76.51±0.79	75.07±0.73	0.200
Neutral detergent fibre	48.30±1.37	52.98±1.44	0.043
Acid detergent fibre	40.11±1.59	44.14±1.64	0.059
Hemicellulose	59.59±1.07	60.82±1.15	0.447
<i>Nutritive value</i>			
Digestible crude protein (%)	8.64±0.13	8.42±0.13	0.246
Total digestible nutrients (%)	68.69±0.92	68.89±0.92	0.881
Nutritive ratio	6.96±0.25	7.17±0.23	0.312
Digestible energy (Mcal/kg)	3.03±0.04	3.04±0.04	0.865
Metabolisable energy (Mcal/kg)	2.48±0.03	2.49±0.03	0.855

[‡]Treatment groups: Cont, Control (animals fed control diet, i.e. basal TMR without replacement) ; ELMG, animals fed TMR with 30% (w/w) CFM replaced with ELM.

Despite that the protein content of the treatment ration containing ELM was little less than that of control ration; however, the nutritive value of both the diets were comparable though numerically better for ELMG diet probably due to similar DM intakes as well as nutrient digestibility for the experimental rations. The results are in agreement with those of Patra *et al.* (2002).

Nitrogen and macro-mineral (Ca and P) balances: Both the groups had positive balances of N, Ca and P; however, balances of N were comparable between the two groups, whereas balance of Ca (g/d) and P (g/d as well as % absorbed) was higher ($P<0.05$) in ELMG compared to Con group (Table 4). The comparable N intake between the dietary groups was the results of similar CP intakes;

Table 4. Balances of nitrogen and some major minerals in sheep fed TMR with partially replaced concentrate feed mixture by Elm leaf meal

Balance of mineral		Parameter	Treatment groups [‡]		P value
			Con	ELMG	
Nitrogen	Intake	(g/d)	19.97±0.38	19.08±0.32	0.092
	Voided	Faeces (g/d)	7.34±0.25	6.40±0.20	0.010
		Urine	6.11±0.26	5.90±0.67	0.770
	Retained	g/d	6.52±0.35	6.78±0.75	0.770
		% intake	32.67±1.67	35.55±3.95	0.514
Calcium	Intake	(g/d)	12.50±0.34	14.28±0.24	<0.001
	Voided	Faeces (g/d)	5.28±0.18	5.35±0.17	0.789
		Urine	2.35±0.25	2.98±0.13	0.045
	Retained	g/d	4.87±0.31	5.95±0.23	0.014
		% intake	38.98±2.57	41.72±1.56	0.378
Phosphorus	Intake	(g/d)	7.39±0.14	7.80±0.13	0.049
	Voided	Faeces (g/d)	2.44±0.08	2.46±0.08	0.863
		Urine	2.22±0.01	2.19±0.02	0.260
	Retained	g/d	2.73±0.11	3.15±0.13	0.026
		% intake	36.84±1.07	40.29±1.24	0.054
		% absorbed	54.95±1.08	58.79±1.09	0.025

[‡]Treatment groups: Cont, Control (animals fed control diet, i.e. basal TMR without replacement); ELMG, animals fed TMR with 30% (w/w) CFM replaced with ELM.

however, lower ($P < 0.05$) fecal excretion in ELMG was probably due to effect of tannins in ELM based diet. Olafadehan *et al.* (2020) reported that concentrate replacement with 250 and 500 g/kg *Daniellia oliveri* foliage reduced fecal N excretion (as g/kg N intake) by 26 and 32%, respectively in goats. Animals of both the dietary groups were in positive N balances which were comparable between the groups, indicating adequate nutritional level of all the animals irrespective of the dietary group. The results of the present study are in close agreement with the reports of Patra *et al.* (2002).

The higher intake and retention of Ca and P in ELMG compared to control diet fed animals were the consequences of high mineral content in ELM and subsequently in the ration. The intakes of Ca and P were even sufficiently higher than the recommended values (Kearl 1982) to support the increase in live weight of animals in both the groups. The greater urinary Ca excretion in ELMG compared to control was probably due to improper Ca : P ratio in the diet, as tree leaves are rich in Ca but poor in P content (Baidya *et al.* 1995). These results are in close agreement with the findings of Pal *et al.* (2010) who evaluated the effect of feeding diets with concentrate feed replaced by iso-nitrogenous leaf meal mixture in growing goats.

Body weight changes: Replacement of CFM with ELM in TMR did not statistically affect weight gains of sheep, though numerically better values were recorded in ELMG group (Table 5). This may be attributed to the higher CP digestibility and mineral retention, and quality of ELM protein which may have not only provided readily available N, amino acids and peptides for microbial growth in the rumen but also supplied valuable amino acids for absorption in the lower gut leading to better growth performance of animals. The results of no significant difference in weight gain are consistent with the report of Pal *et al.* (2010) and

Das *et al.* (2012) who studied the effects of replacing concentrate feed by leaf meal and stylo meal in the diet for goats and heifers, respectively.

Feeding and production economics: The cost of TMR for the group ELMG and Con were ₹ 19.79 and ₹ 22.21 per kg, respectively (Table 5). Reduction in the cost of TMR with the inclusion of ELM which was obviously due to replacement of CFM (costing ₹ 25.53 and ₹ 26.76 per kg for control and ELMG diets, respectively) with cost free ELM at 30% level. Labour cost for collection and processing of Elm foliage was overcome by cost free availability of the ingredient.

The average daily feeding cost per animal followed the similar trend as that of cost of feeds for the dietary groups. Likewise, the feed cost per kg live weight gain (i.e. cost of production) was significantly ($P < 0.01$) lower for ELMG compared to control. Lower daily feeding cost per animal in treatment group was due to lower cost of feed for the group; while cost of production (cost/kg BW gain) was reduced to the tune of 35.84% due to better nutrient utilisation that improved body weight changes in sheep and correspondingly increased the net income from the sale of sheep at the end of the feeding trial. These results are comparable with the findings of Olafadehan *et al.* (2020) reported lower cost/kg BW gain in goats fed diet with concentrate feed replaced with *Daniellia oliveri* foliage.

Elm leaf meal can replace 30% of concentrate feed mixture on weight basis in the total mixed ration of sheep without affecting voluntary intakes, nutrient utilization, nutritive value of diet, N and mineral retention, and body weight gain. So economic concentrate feed mixture can be formulated for sheep using Elm foliage as a cost effective feed resource for higher profitability.

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Table 5. Body weight changes and production economics of sheep fed TMR with partially replaced concentrate feed mixture by Elm leaf meal

Attribute	Treatment groups [‡]		P value
	Con	ELMG	
<i>Body weight changes</i>			
Initial BW (kg)	23.47±0.52	23.39±0.41	0.534
Final BW (kg)	25.09±0.49	25.27±0.42	0.538
Net gain (kg)	1.62±0.23	1.89±0.13	0.169
Gain/day (g)	54.12±7.80	62.87±4.38	0.169
<i>Production economics</i>			
Cost of TMR (₹/kg)	22.21	19.79	–
Daily feeding cost/animal (₹)	20.23±0.05	18.33±0.04	<0.001
Cost/kg BW gain (₹)	446.98±4.78	307.48±3.97	<0.001
Reduction in cost of feed/kg BW gain (%)	0.00	35.84±5.94	–

[‡]Treatment groups: Cont, Control (animals fed control diet, i.e. basal TMR without replacement); ELMG, animals fed TMR with 30% (w/w) CFM replaced with ELM.

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