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Research Article

EFFECT OF FEEDING COMPLETE DIET SUPPLEMENTED WITH FEED ADDITIVES ALONE AND IN COMBINATION ON NUTRITIONAL AND HEPATORENAL FUNCTION TEST PROFILE IN CROSSBRED LAMBS

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ABSTRACT

To augment sheep productivity in temperate regions by improving feed utilization efficiency, the present study was undertaken to assess the effect of supplementation of exogenous fibrolytic enzymes cocktail and *Artemisia absinthium* L. (locally known as Afsanteen) herb alone and in combination as feed additives in oats straw based complete diet on nutritional and hepatorenal organ function test profile in lambs. Crossbred lambs (n=20) were randomly divided into four groups of 5 animals each and were fed for a period of 90 days followed by 6 day metabolism trial. Feed intake was monitored daily and animals were weighed at fortnightly intervals. Animals in all the groups were offered complete feeds based on oats straw- 40 parts, mixed grass hay- 20 parts and concentrate mixture- 40 parts. The complete feeds were supplemented either with exogenous fibrolytic enzymes cocktail (T_1) @ 6 g/kg DM or Afsanteen herb (T_2) @ 4.5% of DM or in combination of the two (T_3), whereas the complete feed without any supplementation served as control (T_0). Blood samples were analysed for various kidney and liver functioning test parameters. Mean apparent digestibility values of OM, net weight gain and average daily gain (ADG) were significantly ($P<0.01$) higher in all feed additives supplemented groups in comparison to control. Supplementation of feed additives had significant effect on overall mean A:G ratio ($P<0.01$) and ALT ($P<0.05$) levels, while significant effect on overall mean serum total proteins was recorded in T_2 and T_3 groups. Mean serum urea nitrogen concentration was significantly ($P<0.01$) lower in groups T_2 and T_3 than T_0. No significant differences were recorded for serum creatinine and uric acid among the treatment groups. It was concluded that supplementation of exogenous fibrolytic enzymes cocktail and *A. absinthium* L. herb alone or in combination to complete feed is possible for intensive rearing of lambs successfully without any adverse effect on hepatorenal organ functioning.

Key words: Complete diet, enzymes, herb, KFT, lambs

In developing countries like India, farmers are forced to utilize crop residues like straws, stovers and other fibrous agro-industrial byproducts to feed their livestock due to constant depletion of grazing lands. However, these feedstuffs have low nutritive value and have complex networks formed by structural carbohydrates and lignin that restrict their efficient utilization. To increase the nutrient availability from such feedstuffs, addition or supplementation of feed additives as exogenous fibrolytic enzymes (EFE) and medicinal plants (herbs) has received considerable attention. The EFE works synergistically with endogenous rumen microbial enzymes and thus could increase the digestion and nutritive value of fibrous diet (Morgavi *et al*., 2000). These EFE when added to the diet improved the feed intake and the energetic value of the ruminant diets (Pinos-Rodriguez *et al*., 2002). Herbal feed additives could influence either the feeding pattern or the growth of favorable microorganisms in the rumen or stimulate the secretion of various digestive enzymes, which in turn may improve the efficiency of utilization of nutrients, resulting in improved productive and reproductive performances (Bakshi and Wadhwa, 2000).

With the demand for organic food and ban on the use of antibiotics/hormones/arsenicals etc. in animal diets, the search for alternative feed additives has become necessary. Among non-antibiotic feed additives, the use of EFE and herbs have ability to augment livestock production performance; but prior to advocation, information regarding their effect on physiological health status need to be investigated. In this context, the current study was carried out to assess the effect of feeding oats straw based complete diet supplemented with EFE cocktail and *Artemisia absinthium* L. (Afsanteen) herb as feed additives alone and in combination on the nutritional and hepatorenal function test profile in crossbred lambs.

MATERIALS AND METHODS

Animal Management and Experimental Feeding: Twenty growing crossbred male lambs (4-6 months age

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and 11.58±0.01 kg mean body weight) of uniform conformation procured from sheep unit of Mountain Research Centre for Sheep and Goat, Faculty of Veterinary Sciences and Animal husbandry, SKUAST-Kashmir, were randomly distributed to four groups having five animals in each. The lambs were stall fed individually for a period of 90 days followed by 6 day metabolism trial. Animals in all the groups were offered complete feeds to meet their nutrient requirements as per ICAR (2013). The complete feeds were based on oats straw (40 parts), mixed grass hay (20 parts) and concentrate mixture (40 parts) without (T_0) or with feed additives alone as EFEs cocktail (T_1) @ 6 g/kg DM or Afsanteen herb (T_2) @ 4.5% of DM and in combination (T_3), where the animals of group T_0 served as control maintained on the complete ration without any supplementation. The concentrate mixture consisted of crushed maize (27.5%), wheat bran (12.5%), deoiled rice bran (10%), mustard oilcake (15%), soybean (28.5%), molasses (2.5%), mineral mixture (1.5%), salt (1%) and urea (1.5%).

All lambs were kept under uniform management conditions, housed in well ventilated, hygienic and protected sheds with batten floor, and appropriate facilities for individual feeding and watering. All the animals were dosed for ecto- and endo-parasites, and vaccinated against prevalent contagious diseases before the start of study which was conducted only after approval of Institutional Animal Research and Ethical Committee. Proper management procedures were adopted during the entire period of the study.

Collection and Analysis of Blood: Blood samples were collected in the morning before feeding and watering from jugular vein at the start and subsequently at monthly intervals of the experimental period from each animal. Serum was separated and analyzed for total proteins, albumins, alanine aminotransferase (ALT), urea nitrogen, creatinine and uric acid on Photometer-5010V5$^+$ semi-auto biochem analyzer of Robert Riele INC (Berlin, Germany) using standard kits manufactured by DiaSys Diagnostics India Pvt. Ltd., Navi Mumbai, India. Serum globulins were obtained by subtracting the serum albumin content from serum total proteins. Serum albumin: globulin ratio was then calculated.

The data generated were subjected to statistical analysis using Duncan's multiple range test (Snedecor and Cochran, 1994) in a completely randomized block design.

RESULTS AND DISCUSSION

Nutrient Utilisation and Body Weight Gains: The results of nutritional studies have been summarized in Table 1. The mean apparent digestibility values of OM were significantly (P<0.01) higher in groups T_1, T_2 and T_3 in comparison to group T_0; the groups supplemented with the feed additives were statistically at par. The overall mean CP digestibility was found to be significantly (P<0.01) higher in T_3 in comparison to T_0 group, while T_1 and T_2 groups were comparable to each other as well as to T_0 and T_3 groups. Improvement in the digestibility values of nutrients due to feed additives supplementation might probably be due to enhanced gross activity of rumen microflora on inclusion of different feed additives in the ration of animals (Bhatt, 2015). These results of the present study are supported by the findings of Qiao *et al.* (2013).

Intake of digestible nutrients was numerically improved by feed additives when supplemented singly and had statistical significance (P<0.01) when supplemented in combination. This could be attributed to the higher nutrient digestibility in respective feed additives supplemented groups. These results of the present study find support from the work of Thakur *et al.* (2010) for exogenous enzyme and Gupta *et al.* (2005) for herb supplementation. Significantly (P<0.01) higher net weight gain and ADG were recorded in feed additives supplemented groups in comparison to un-supplemented group. The higher net weight gain and ADG could possibly be due to better absorption and utilization of nutrients on account of supplementation of feed additives.

Table 1
Nutritional profile of crossbred lambs in different treatment groups

Attribute	Parameter	Treatment groups			
		T_0	T_1	T_2	T_3
Nutrient digestibility (%)	OM*	$60.35^a \pm 2.01$	$67.32^b \pm 1.94$	$67.63^b \pm 2.79$	$69.76^b \pm 1.88$
	CP**	$60.01^a \pm 2.94$	$64.36^{ab} \pm 2.90$	$68.50^{ab} \pm 2.12$	$71.16^b \pm 1.73$
Digestible nutrient intake (g/d)	DCPI**	$87.38^a \pm 6.78$	$101.08^{ab} \pm 5.70$	$113.36^{ab} \pm 6.20$	$119.82^b \pm 6.13$
	TDNI**	$515.51^a \pm 28.26$	$616.22^{ab} \pm 24.56$	$657.52^{ab} \pm 40.86$	$687.95^b \pm 38.36$
Growth performance	Net weight gain** (kg)	$6.92^a \pm 0.51$	$10.58^b \pm 0.32$	$10.92^b \pm 0.39$	$12.30^b \pm 0.44$
	ADG** (g/d)	$77.78^a \pm 7.61$	$117.56^b \pm 5.78$	$121.33^b \pm 5.29$	$136.67^b \pm 8.73$

The means across the rows with different superscript differ significantly (*P≤0.05; **P≤0.01)

Tirado-Estrada *et al*. (2011) also reported significantly higher ADG in lambs fed corn stover based diets supplemented with fibrolytic enzyme mixtures in comparison to those fed un-supplemented diet. Likewise, Abdelhamaid *et al*. (2011) observed significantly higher total body weight and daily body gain in goats supplemented with medicinal herbs in their diets.

Liver Function Test Profile: Table 2 depicts the results pertaining to the hepatic function attributes of crossbred lambs. Supplementation of feed additives had significant effect on overall mean A:G ratio (P<0.01) and ALT (P<0.05) levels, while significant effect of period on overall means serum total proteins was recorded. The mean values of serum total protein, A:G ratio and ALT obtained in the present study were within normal physiological ranges (Kaneko *et al*., 1997). Higher values for total protein while lower values for A:G ratio in feed additive supplemented groups compared to un-supplemented group were recorded. Statistical significance could be observed only for A:G ratio which could be attributed to either higher dietary protein intake resulting in higher nutritional plane or higher immune response causing reduction in gastro-intestinal parasitic burdens. Non-significant increase in serum total protein was also reported by Gupta *et al*. (2006) in heifers fed mixture of different herb, and Peters *et al*. (2015) in lactating dairy cows fed diets supplemented with exogenous enzymes. Dietary supplementation of the feed additives in combination had significant influence on the serum level of hepatic enzyme ALT. The values of the hepatic enzyme were lower in the feed additives supplemented groups in comparison to un-supplemented group with the lowest values for herb-enzyme supplemented animals. These changes in the levels of hepatic enzyme, especially in group supplemented with the feed additives in combination suggest functional property of the essential oils present in Afsanteen herb which had hepato-protective activity. The hepato-protective action of Afsanteen herb has been already documented by Romero *et al*. (2005). The results of the present study are comparable with the findings of Rivero and Salem (2015) for fibrolytic enzymes, and Niwas *et al*. (2012) for herb, who also reported lower ALT activity by feed additive supplementation.

Kidney Function Test Profile: The results pertaining to the kidney function attributes of crossbred lambs are presented in Table 3. Groups T_2 and T_3 had significantly lower (P<0.01) mean serum urea nitrogen concentration as compared to group T_1, however, there was no significant (P>0.05) changes in serum creatinine and uric acid concentrations among the groups. Across periods, a significant steady increase was recorded in overall means for serum urea nitrogen (P<0.05) as well as serum creatinine and uric acid (P<0.01).

Decreased serum urea nitrogen levels in Afsanteen herb (T_2 and T_3) fed group might probably be due to efficient utilisation of dietary proteins due to the herb as feed additive, although the values in all the groups were within normal range (Kaneko *et al*., 1997). Slower rate of dietary protein degradation along with better utilization of generated ammonia in the rumen causing lower nitrogen wastage which was represented in terms of

Table 2
Liver function test profile of crossbred lambs at monthly intervals

Attribute	Period (days)	Treatment groups				Mean±SE	Sig.
		T_0	T_1	T_2	T_3		
Serum total proteins (g/dL)	0	6.57±0.21	6.34±0.09	6.50^{A}±0.09	6.52^{A}±0.10	6.48^{A}±0.05	P*
	30	6.50±0.24	6.40±0.17	6.68^{AB}±0.15	6.66^{AB}±0.14	6.56^{A}±0.07	
	60	6.50±0.29	6.68±0.19	6.80^{AB}±0.16	6.96^{AB}±0.17	6.73^{AB}±0.10	
	90	6.55±0.27	6.80±0.19	6.94^{B}±0.16	7.12^{B}±0.18	6.85^{B}±0.12	
	Mean±SE	6.53±0.02	6.55±0.09	6.73±0.07	6.81±0.09	6.66±0.05	
Serum A:G ratio	0	1.10±0.08	1.18±0.07	1.15±0.05	1.15±0.03	1.14±0.02	T**
	30	1.31±0.11	1.21±0.09	1.07±0.05	1.10±0.06	1.17±0.05	
	60**	1.31^{b}±0.10	1.20^{ab}±0.05	1.07^{ab}±0.06	1.02^{a}±0.06	1.15±0.06	
	90**	1.29^{b}±0.05	1.24^{ab}±0.08	1.03^{ab}±0.05	1.02^{a}±0.06	1.14±0.07	
	Mean**±SE	1.25^{b}±0.05	1.21^{ab}±0.03	1.08^{a}±0.03	1.07^{a}±0.03	1.15±0.03	
ALT (IU/L)	0	24.03±0.75	24.06±0.54	24.60^{B}±0.62	25.32^{B}±0.90	24.50±0.30	T*
	30	23.65±0.86	23.46±0.59	23.30^{AB}±0.76	21.22^{A}±1.06	22.91±0.57	
	60*	24.70^{b}±1.73	23.06^{ab}±0.58	20.98^{aA}±1.04	20.44^{aA}±1.02	22.29±0.98	
	90*	25.00^{b}±1.66	24.26^{b}±1.33	22.54^{abAB}±1.14	20.14^{aA}±0.87	22.98±1.07	
	Mean*±SE	24.34^{b}±0.31	23.71^{ab}±0.40	22.85^{ab}±0.52	21.78^{a}±0.65	23.17±0.41	

The means across the rows with different lower case superscript differ significantly (*P≤0.05; **P≤0.01)
The means across the columns with different uppercase superscripts differ significantly (P≤0.05)

Table 3
Kidney function test profile of crossbred lambs at monthly intervals

Attribute	Period (days)	Treatment groups				Mean±SE	Sig.
		T_0	T_1	T_2	T_3		
Serum urea nitrogen	0	34.47^{A}±0.90	34.01±0.62	32.54±0.74	33.13±0.83	33.49^{A}±0.39	T**
	30	35.84^{AB}±1.26	34.75±0.98	33.42±0.86	33.61±0.92	34.33^{A}±0.50	
	60	37.08^{AB}±1.36	34.91±1.21	33.36±1.57	32.92±1.15	34.43^{A}±0.71	P*
	90	38.46^{B}±1.47	36.19±1.37	35.50±1.08	34.85±1.05	36.13^{B}±0.64	
	Mean**±SE	36.46^{b}±0.85	34.97^{ab}±0.45	33.71^{a}±0.63	33.63^{a}±0.43	34.60±0.30	
Serum creatinine	0	1.32^{A}±0.02	1.35^{A}±0.02	1.34^{A}±0.03	1.33^{A}±0.03	1.34^{A}±0.01	P**
	30*	1.46^{abAB}±0.03	1.46^{abB}±0.01	1.50^{bB}±0.01	1.43^{aB}±0.03	1.46^{B}±0.01	
	60	1.51^{B}±0.06	1.51^{B}±0.02	1.46^{B}±0.01	1.44^{B}±0.03	1.48^{B}±0.02	
	90**	1.52^{abB}±0.07	1.59^{bC}±0.03	1.35^{aA}±0.03	1.52^{abB}±0.03	1.49^{B}±0.03	
	Mean±SE	1.45±0.05	1.48±0.05	1.41±0.04	1.43±0.04	1.44±0.01	
Serum uric acid	0	1.13^{A}±0.01	1.16^{A}±0.04	1.08^{A}±0.07	1.11^{A}±0.06	1.12^{A}±0.03	P**
	30	1.23^{B}±0.03	1.25^{AB}±0.04	1.19^{AB}±0.03	1.20^{AB}±0.05	1.22^{B}±0.02	
	60	1.25^{B}±0.02	1.29^{B}±0.04	1.24^{B}±0.02	1.22_{AB}±0.05	1.25^{BC}±0.02	
	90	1.34^{C}±0.01	1.30^{B}±0.04	1.26^{B}±0.03	1.32^{B}±0.07	1.30^{C}±0.02	
	Mean±SE	1.24±0.04	1.25±0.03	1.19±0.04	1.21±0.04	1.22±0.01	

Means across the rows with different lower case superscript differ significantly (*P≤0.05; **P≤0.01);
Means across the columns with different uppercase superscripts differ significantly (P≤0.05)

lower serum urea levels in herb supplemented groups. Across the periods, significant rise in serum urea N level indicated higher intake of protein and energy due to higher feed intake. These findings are in accordance to those reported by Ganai *et al*. (2012) and Niwas *et al*. (2012) for herb and El-Kady *et al*. (2006) for enzyme supplementation.

Metabolism of creatine in muscles results in creatinine production, the level of which in serum is used as an index of turnover of the protein pool in ruminants (Turner *et al*., 2005) but is no condition by the diet, i.e. increase in protein intake has no effect on plasma creatinine concentration (Rivero and Salem, 2015). In the present study, levels of serum creatinine and uric acid in all the groups were within the normal ranges as reported for healthy sheep (Kaneko *et al*., 1997); however, the values were numerically lower in groups fed diets supplemented with Afsanteen herb alone and in combination with EFE in comparison to un-supplemented group, indicating that secondary compounds (EO's and sesquiterpene lactones) present in the Afsanteen herb had no adverse affects on glomerular filtration, thus safe for renal functioning. Mahgoub *et al*. (2008) also did not observe any changes in serum creatinine when provided feeds that contained phenols and condensed tannins to sheep. These findings in the present study are in close agreement to those reported by El-Kady *et al*. (2006) and Rivero and Salem (2015) for enzyme supplementation in buffalo calves and sheep, respectively and Randhawa *et al*. (1995) for herb supplementations.

It is concluded that EFE cocktail as well as Afsanteen herb could be supplemented alone or in combination as feed additives for improving efficiency of feed utilization to raise lambs intensively without any adverse effect on hepato-renal functioning.

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