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Research Article

EFFECT OF FEEDING COMPLETE DIET SUPPLEMENTED WITH EXOGENOUS FIBROLYTIC ENZYME COCKTAIL OR *ARTEMISIA ABSINTHIUM* L. HERB ALONE AND IN COMBINATION ON ENERGY METABOLIC PROFILE OF LAMBS

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ABSTRACT: To augment sheep productivity in temperate regions by improving feed utilization efficiency, the present study was planned to assess the effect of supplementation of exogenous fibrolytic enzyme cocktail and *Artemisia absinthium* L. (locally known as Afsanteen) herb alone and in combination as feed additives in oats straw based complete diet on energy metabolic profile in crossbred lambs. Twenty crossbred lambs were randomly divided into four groups of 5 each and were fed individually for a period of 90 days. 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 alone with exogenous fibrolytic enzyme cocktail (T_1) @ 6 g/kg DM or Afsanteen herb (T_2) @ 4.5% of DM and combination of the two feed additives (T_3), whereas the complete feed without any supplementation served as control (T_0).Blood monitoring was carried out at the start and subsequently at monthly intervals of the experiment. Blood glucose was estimated in whole blood immediately after collection and rest were subjected to serum harvesting to analyze for non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) levels. There were no significant differences for overall blood glucose, serum NEFA and BHB levels among the treatment groups while significant (P<0.01) effect of period were recorded. It may be concluded that supplementation of exogenous fibrolytic enzyme cocktail and *A. absinthium* L. herb alone or in combination to complete feed is possible for intensive rearing of lambs without any adverse effect on energy metabolic profile.

Key words: Complete diet, Energy, Enzyme, Herb, Sheep.

INTRODUCTION

Crop residues like straws, stovers and other fibrous agro-industrial byproducts form the bulk of ration for ruminant livestock in developing countries like India (Rajamma *et al*. 2014). However, these feedstuffs have complex links which limit the degradation of nutritional compounds and restricts their efficient utilization contributing to their poor digestibility and limited energy availability to the animal causing a large excretion of nutrients (Tang *et al*. 2013). This necessitates finding ways for optimizing the use of these crop residues and poor-quality roughages. One option is the addition or supplementation of feed additives to increase the nutrient availability from these available feedstuffs. Among which exogenous fibrolytic enzymes (EFE) and medicinal plants (herbs) have received considerable attention.

Artemisia absinthium L. (Family : Asteraceae) has a long-standing traditional use for various indications. The Egyptians used it as an antiseptic, a stimulant and tonic, and as a remedy for fevers and menstrual pains. Hippocrates recommended it as a cure for jaundice. Pliny's 'Historica Naturalis' describes the extract of this plant is having a long-standing benefit against gastro-intestinal worms. In the Middle Ages, the plant was used to exterminate tapeworm infestations while leaving the human host uninjured. Paracelsus considered it as a

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stomachic, anthelmintic herb which also acts as prophylaxis against sea-sickness (European Medicine Agency 2009).

EFE in the ruminant diet have been reported to improve the fiber digestibility and their use may become common because they do not cause any negative effect on animal health and performance (Hussain *et al*. 2008). 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 nutrient utilization and improve productive performances (Bakshi and Wadhwa 2000). Glucose metabolism has a close association with hepatic ketogenesis and lipid metabolism in liver, where glucose metabolism is integrated with ketone bodies, Non esterified fatty acids (NEFA) and volatile fatty acids metabolism (Huntington and Eisemann 1988). Earlier studies have focused impact of dietary regime, dietary composition or some materials relative to glucose production infusion on energy metabolism in pregnant or lactating ruminant animals (Kraft *et al*. 2009, Kristensen *et al*. 2010), but only a scanty literature is available regarding the effect of feed additive supplementation on energy metabolism in growing ruminants. In this context, the present study is aimed to investigate the effect of supplementation of feed additives as EFE cocktail and *Artemisia absinthium* L. (locally known as 'Afsanteen') alone and in combination on the energy metabolic profile in terms of blood glucose, serum NEFA and Beta hydroxybutyrate (BHB) in crossbred lambs.

MATERIALS AND METHODS

Animal management and experimental feeding

Twenty growing crossbred male lambs (4-6 months age 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, India, were randomly distributed to four groups having five animals in each. The lambs were stall fed individually for a period of 90 days. Animals in all the groups were offered complete feeds to meet their nutrient requirements as per ICAR (2013). Exogenous Fibrolytic Enzyme cocktail (Allenzimix -EP of Mitushi Pharma) was used as feed additive. 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 EFE cocktail (T_1) @ 6 g/kg DM or *Artemisia absinthium* L. (Afsanteen) herb (T_2) @ 4.5% of DM and in combination (T_3). The animals of group T_0 served as control and maintained on the complete ration

Table 1. Energy metabolic profile in crossbred lambs at monthly intervals.

Attribute	Period (days)	Treatment groups				
		T_0^{*}	T_1^{**}	T_2^{**}	T_3^{**}	Mean**±SE
Blood glucose (mg/dL)	0	45.75^{A}±3.06	42.40^{A}±1.86	43.20^{A}±1.59	41.80^{A}±1.59	43.16^{A}±0.97
	30	49.00^{AB}±1.08	47.40^{AB}±1.43	48.00^{AB}±1.22	48.60^{AB}±1.36	48.21^{B}±0.61
	60	49.50^{AB}±0.87	50.60^{B}±1.21	50.20^{B}±1.24	49.80^{B}±1.88	50.05^{BC}±0.65
	90	52.00^{B}±1.22	54.40^{B}±2.38	50.40^{B}±1.94	52.20^{B}±2.24	52.26^{C}±1.01
	Mean±SE	49.06±1.16	48.70±1.42	47.95±1.31	48.10±1.59	—
NEFA (μM/L)	0	199.25^{B}±9.81	209.80^{C}±5.45	208.20^{C}±3.73	215.40^{C}±5.12	208.63^{D}±3.03
	30	186.25^{AB}±1.75	189.60^{B}±1.81	191.60^{B}±3.53	192.80^{B}±2.85	190.26^{C}±1.35
	60**	184.25^{bAB}±2.17	169.20^{aA}±1.56	175.40^{abA}±4.39	175.20^{abA}±3.40	175.58^{B}±1.89
	90**	177.50^{bA}±3.12	162.40^{abA}±3.66	161.60^{aA}±3.85	159.80^{aA}±3.56	164.68^{A}±2.28
	Mean±SE	186.81±2.09	182.75±1.51	184.20±3.57	185.80±2.50	—
BHB (μM/L)	0	58.87^{B}±2.49	63.34^{C}±2.15	62.66^{C}±1.75	63.62^{D}±1.85	62.29^{D}±1.02
	30	54.50^{AB}±1.20	55.48^{BC}±2.17	56.52^{BC}±1.53	57.84^{C}±0.86	56.17^{C}±0.77
	60	53.67^{AB}±1.53	50.76^{AB}±1.34	50.42^{AB}±1.25	52.56^{B}±1.02	51.76^{B}±0.66
	90*	51.57^{bA}±1.41	46.32^{aA}±2.36	45.42^{aA}±1.46	46.94^{abA}±1.07	47.35^{A}±0.93
	Mean±SE	54.66±0.80	53.98±1.76	53.76±1.40	55.24±0.87	—

The means across the rows with different lower-case superscript differ significantly (*P<0.05; **P<0.01).
The means across the columns for each parameter with different uppercase superscripts differ significantly (*P<0.05; **P<0.01).

without any supplementation. Whole aerial parts of the plant including leaves, twigs and stem of *A. absinthium* L. were dried and grinded in a willey mill to pass through 1 mm screen for being used as feed additive in the feeding trial. 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. Proper ethical care and management procedures were adopted during the entire period of the study.

Collection and analysis of blood

To corroborate the results of nutritional findings, periodic monitoring of blood parameters (energy metabolic profile) was carried out. The blood samples were taken from all the animals at the start (day 0) and subsequently at monthly intervals (day 30, 60 and 90) of the experimental trial period by puncturing the jugular vein. Blood glucose was estimated in whole blood immediately after collection using SD-Code free Blood Glucose Meter (SD Biosensor Healthcare Pvt. Ltd., Gurgaon, Haryana, India). The samples were then centrifuged (at 2000 × g for 10 min at 15°C) within 4 h after collection to obtain serum, which was immediately stored in eppendorf tubes at –20°C until analysis. The biochemical blood components were measured by different colorimetric techniques using a semi-auto biochem analyzer for clinical chemistry (Photometer-5010V5$^+$ of Robert Riele INC, Berlin, Germany) and different commercial kits in accordance with International Federation of Clinical Chemistry (IFCC) recommendations. For NEFA, a commercial kit (Dia Sys Diagnostics Pvt. Ltd., Navi Mumbai, India) was used. BHB was analyzed with commercial kit (Transasia Bio-medicals Ltd.) using an enzymatic UV method.

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

The results pertaining to metabolic profile used to monitor the energy status in terms of blood glucose, serum NEFA and BHB levels of crossbred lambs fed complete diets supplemented with fibrolytic enzyme cocktail and Afsanteen herb alone and in combination are presented in Table 1.There was no significant ($P>0.05$) effect of any feed additive supplemented either alone or in combination but highly significant ($P<0.01$) effect of period on overall means of blood glucose, serum NEFA as well as BHB levels. Across periods, a significant steady increase for blood glucose and a significant steady decrease for NEFA and BHB were observed in all the treatment groups.

In the present study, the values of blood glucose concentration in all the groups varied non-significantly and were within the normal range (Kaneko *et al.* 1997). However, the values were numerically lower in lambs of feed additive supplemented groups in comparison to control group probably due to stimulated insulin production leading to increased glucose metabolism in tissues by feeding the feed additives. Insulin enhances lipogenesis by promoting uptake of glucose and free fatty acid into peripheral tissues. In support of the present findings, Noori and Al-Waili (1986), and Cefalu *et al.* (2008) also reported hypoglycaemic activity of *Artemisia absinthium* and suggested its use for management of diabetic patients. No change in the level of blood glucose in Holstein steers fed diets supplemented with peppermint, clove and lemongrass has been reported by Hosoda *et al.* (2006). Likewise, concentration of serum glucose in Black Bengal goats remained un-altered by supplementation of plant additives containing high contents of saponins (*Acacia concinna* pods) and essential oils (*Syzygium aromaticum* buds) (Mandal *et al.* 2014). No significant effect of enzyme supplementation on concentration of plasma glucose has also been observed by Vahora and Pande (2006) in dairy cows.

NEFA and BHB are released in the blood plasma when adipose tissue is mobilized to supply metabolic needs of the animal, primarily the need for energy; although there levels in the blood of ruminants are small, but are important factors in determining caloric homeostasis of the body. In the present study, the results revealed no significant effects of the feed additives supplementation on overall serum NEFA and BHB levels in comparison to un-supplemented (control) group, which could be attributed to non-significant effect of treatments on feed intake (639.96±81.46, 660.79±84.47, 668.49±89.93 and 707.78±85.16 g/d forT_0, T_1, T_2 and T_3, respectively) or adequate levels of metabolisable energy (1.99±0.06, 2.22±0.07, 2.21±0.09 and 2.29±0.05 Kcal/g DM for T_0, T_1, T_2 and T_3, respectively) and TDN (59.02±1.93, 65.30±1.89, 66.05±2.66 and 67.91±1.78 % for T_0, T_1, T_2 and T_3, respectively) content of all the diets fulfilling the energy requirements of the animals in all the groups thereby restricting lipid mobilization; although, the values being numerically lower for the animals fed diets supplemented with feed additives alone as well as in combination presumably due to increased metabolism of glucose that might have depressed the fatty acid mobilization in peripheral adipose tissues in the respective groups, so that less free fatty acid became available for hepatic uptake and oxidation. Use of EFE improves the energy status of ruminants by reducing plasma concentrations of NEFA and BHB, indicating reduction in mobilization of fat from adipose tissue (Dean *et al*. 2013). Besides, herbs or their active component(s) modify fat metabolism in the body by inhibiting the hepatic fatty acid synthetase activity, which is the key regulatory enzyme in endogenous fat synthesis (Lee *et al*. 2004). Treacher *et al*. (1976) reported that glucose infusion via jugular vein into the normal fed cows in early lactation for 48 h increased plasma glucose concentration associated with the decreased concentrations of BHB and NEFA. In agreement to the present findings, no effect of herb feeding treatments on the concentrations of NEFA in plasma was also observed by Hosoda *et al*. (2006) in Holstein steers. Likewise, Peters *et al*. (2015) observed no effects of exogenous fibrolytic enzymes supplementation on serum BHB in early and mid-lactation Holstein cows.

CONCLUSION

It can be concluded that EFE cocktail as well as Afsanteen herb could be supplemented alone as well as in combination as feed additives in complete diet for improving feed utilization efficiency to raise lambs intensively without any adverse effect on energy metabolic profile.

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