RESEARCH ARTICLE

Serum Lipid Profile in Sheep Fed Diet Incorporated with Feed Additives

Yasir A Beigh[1]*, Abdul M Ganai[1], Showkeen Muzamil[2], Danish M Mir[1], Haidar A Ahmad[1], Shahid H Mir[3]

Abstract

A feeding trial to study the supplementation effect of exogenous fibrolytic enzymes (EFE) cocktail and wormwood herb on serum lipid profile was conducted for 90 days in twenty crossbred lambs that were divided into four equal groups viz. T_0 (Control) and T_1, T_2 and T_3 (treatment groups). T_0 received total mixed diet (TMR) without any supplementation, while in treatments groups TMR was supplemented either with EFE cocktail alone @ 0.60% (T_1) or wormwood herb alone @ 4.50% (T_2) and in a combination of the two feed additives (T_3). At the end of the feeding trial, 6 days of digestibility trial was carried out to assess the supplementation effect in vivo on nutritional parameters and serum total lipids and different lipoprotein fractions were carried out at the start (0 d) and subsequently at monthly intervals of the experiment (30, 60 and 90 d). Nutrient digestibility, content as well intake were significantly higher in groups fed diets supplemented with the feed additives either alone (p <0.05) or in-combination (p <0.01). No significant effect was observed on the total cholesterol level at any point of evaluation, and on total triglycerides level-up to 30 d, but significant reduction was noted in T_2 at 60 (p <0.05) and 90 (p <0.01) days as compared to T_1. Significant (p <0.01) effect on HDL level was evident as elevated in T_2 while reduced in T_1 at day 60 and 90. In contrast, LDL values were reduced in T_2 and elevated in T_1 group at day 30 (p <0.05), 60 and 90 (p <0.01). VLDL levels did not change among the groups up to 30 d, whereas reduced at day 60 (p <0.05) and 90 (p <0.01) in T_2 as compared to T_1. Lowest (p <0.01) AI value was observed in the T_2 group at day 90 compared to other groups. It was concluded that dietary incorporation of wormwood herb as a feed additive for sheep improved nutritional status along with health beneficial effects when supplemented alone.

Keywords: Exogenous enzymes, Herb, Lipid, Sheep, Total mixed diet.

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Introduction

The major constraints in today's livestock sector are high feed cost and low quality of available feed resources, especially in tropical developing countries. Over the years animal nutritionists have developed various physical, chemical and biological methods to overcome the problems associated with livestock feedstuffs. In the recent past, exogenous fibrolytic enzymes (EFE) have been developed to increase feed digestibility in ruminants (Beauchemin *et al.,* 2003; Colombatto *et al.,* 2003). It has been demonstrated that EFE work in synergy with the endogenous rumen microbial enzymes to enhance the digestibility and nutritive value of high fibrous diet (Morgavi *et al.*, 2000), thereby increasing the economic benefits for the farmer.

Along with fibrolytic enzymes, plants, and their derivative (herbs) have been used in order to increase feed utilization efficiency in animals. *Artemisia absinthium* is one of them. *Artemisia absinthium* L. commonly known as wormwood belongs to the Asteraceae family and grows naturally on non-cultivated, arid ground, on rocky slopes and at the edge of footpaths and fields as a perennial herb with fibrous roots. Wormwood is a highly valuable medicinal plant, distributed mainly in the temperate zones of Asia, Europe, and North America. In India, it is naturally distributed in the Himalayan region across Jammu and Kashmir. Wormwood has great importance, widely used as an antiseptic, anthelmintic, and for the treatment of cardiovascular diseases including atherosclerosis and other cerebrovascular diseases in humans related to hyperlipidemia (Beigh and Ganai, 2017). *A. absinthium* ethanolic extract (500, 1000 mg/kg) have potent antihyperlipidemic activity in high cholesterol diet-induced

[1]Division of Animal Nutrition, Faculty of Veterinary Sciences & Animal Husbandry, Shuhama, Alusteng, Srinagar, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

[2]Division of Veterinary Biochemistry, Faculty of Veterinary Sciences & Animal Husbandry, Shuhama, Alusteng, Srinagar, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India

[3]Dairy Cattle Nutrition Division, National Dairy Research Institute, Karnal, Haryana, India

Corresponding Author: Yasir A Beigh, Division of Animal Nutrition, Faculty of Veterinary Sciences & Animal Husbandry, Shuhama, Alusteng, Srinagar, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Jammu & Kashmir, India Phone: +91-9419024721; email: vetyasir1@gmail.com

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hyperlipidemia models in rabbits (Daradka *et al*., 2014). The objective of this study was to determine the supplementation effects of exogenous fibrolytic enzymes and *Artemisia absinthium* on the serum lipid profile of sheep.

Materials and methods

The EFE mix (ALLENZIMIXEP, supplied by Mitushi Pharma, Ahmedabad, Gujarat, India) contained enzymes as cellulase (800000 IU/g), phytase (400000 IU/g), β glucanase (450000 IU/g), xylanase (400000 IU/g) and pectinase (350000 IU/g). The plant (wormwood herb) was obtained from the Aharbal area of southern Kashmir valley, harvested in last week of June 2016. Leaves and small twigs were stripped from the stems, dried, crumbled, sieved through a 4.75 mm mesh screen, and stored over a desiccant at 4°C until used.

The experimental protocol was approved by the local Ethics Committee (National Advisory Committee for Laboratory Animal Research, NACLAR, 2004). The principles of animal protection were strictly followed. Experiments under *in vivo* conditions were performed on twenty growing crossbred (Fec-B carrying gene×Hampshire) male lambs (4-6 months age with 11.58 ± 0.01 kg mean body weight) of uniform conformation, randomly allotted to four groups of five each. The lambs were stall-fed individually for a period of 90 days. The feed contained oats straw–40 parts, mixed grass hay- 20 parts and concentrate mixture–40 parts and served as the control diet (T_0). The experimental diets were total mixed ration (TMR) to which the fibrolytic enzyme cocktail was supplemented @0.60% DM (T_1), wormwood herb @4.50% (T_2) and combination of the two additives (T_3). At the time of feeding, the feed additives were first hand-mixed with a concentrated mixture, then with chopped straw to obtain the total mixed diets.

All the lambs were kept under uniform management conditions of feeding and watering. The animals were dosed and vaccinated before the start of the study. After the completion of 90 days feeding experiment, six days digestibility trial was conducted using metabolic cages during which records of feed intake, orts, and faeces voided were maintained. Samples of faeces and residue leftover collected during the digestibility trial daily (approximately 10% of total amounts) were later pooled for each animal, dried, ground and stored until chemical analyses.

Collection and Chemical Analysis of Blood

Blood samples (~ 8 mL) were collected from all the animals at the start (day 0) and subsequently at 30, 60 and 90 days by puncturing the jugular vein. The samples were then centrifuged (at 2000 × g for 10 min at 15°C) within 4 hours after collection to obtain serum, which was immediately stored in Eppendorf tubes at –20°C until analysis. The concentration of total cholesterol (TC), total triglycerides (TG) and high-density lipoprotein (HDL) in serum were measured enzymatically by using semi-auto biochem analyzer (Photometer–5010V5$^+$, Robert Riele INC, Berlin, Germany) and commercial kits (DiaSys Diagnostics Pvt. Ltd., Navi Mumbai, India) in accordance with International Federation of Clinical Chemistry (IFCC) recommendations. Very low-density lipoprotein (VLDL) was estimated using Friedewald *et al*. (1972) method. Low-density lipoprotein (LDL) and Atherogenic index (AI) were measured by using the equations as below:

$$LDL = TC - (HDL + VLDL)$$

$$AI = (TC - HDL)/HDL$$

For determination of nutritional profile, the samples of experimental total mixed diets, refusals and faeces were thawed, dried and chemically analyzed as per AOAC (2005) for dry matter, total ash, crude protein, and ether extract. Digestible nutrient contents of the diets in terms of digestible crude protein (%DCP) and metabolizable energy (ME KJ/g) were determined from the chemical composition and average apparent nutrient digestibility coefficients.

Statistical Analysis

The data were subjected to two-way analysis of variance in a completely randomized design with a model containing treatment as well as period effects using SPSS version 20.0 software for windows. Duncan's multiple range test was used to detect significant differences among the means.

Results and discussion

In Vivo Nutrient Digestibility Study

The effect of supplementation of EFE and wormwood herb in the diet of lamb on *in-vivo* nutrient digestibility studies is given in Table 1. There was a significant increase (p <0.01) in digestibility of nutrients (OM, CP), content (DCP%, ME) and intake of digestible nutrients per day (DCPI and MEI) in T_3; however the increase was only at p <0.05 level in T_1 and T_2 compared to control group. Improvement in nutrient digestibility represents the efficacy of the feed additives in augmenting nutrient utilization. The ability of EFE to loosen lignocellulosic bonds in dietary fiber (Gado *et al*., 2011) and essential oils (EO) of wormwood herb to stimulate the digestion processes (Judzentiene, 2016) could have been responsible for higher nutrient digestibility observed in the present study.

Serum Lipid and Lipoprotein Profile

No significant effect was observed on the total cholesterol level in any of the three treatment groups (T_1, T_2, and T_3) upon supplementation of EFE and wormwood; however, increase in total cholesterol from day 0 to day 90 was significant statistically (p <0.01) in T_1 and only numerically in rest of the groups. Likewise, there was no significant effect of supplementation of EFE and wormwood on total triglycerides (TG) level at 30 days, but significant reduction

Table 1: Nutritional profile of lambs fed diets incorporated with feed additives

Attribute	*Dietary treatments‡*			
	T0	*T1*	*T2*	*T3*
	Nutrient digestibility (%)			
Organic matter*	$60.35^{a} \pm 2.01$	$67.34^{ab} \pm 1.94$	$67.65^{ab} \pm 2.79$	$69.79^{b} \pm 1.88$
Crude protein*	$60.01^{a} \pm 2.94$	$64.38^{ab} \pm 2.90$	$68.60^{ab} \pm 2.12$	$71.26^{b} \pm 1.73$
	Digestible nutrient content of diets			
DCP (%)*	$9.98^{a} \pm 0.49$	$10.71^{ab} \pm 0.48$	$11.44^{ab} \pm 0.35$	$11.89^{b} \pm 0.29$
ME (KJ/g)*	$8.31^{a} \pm 0.24$	$9.28^{ab} \pm 0.31$	$9.24^{ab} \pm 0.40$	$9.60^{b} \pm 0.23$
	Digestible nutrient intakes			
DCPI (g/d)*	$87.38^{a} \pm 6.78$	$101.18^{ab} \pm 5.70$	$113.85^{b} \pm 6.21$	$120.42^{b} \pm 6.15$
MEI (MJ/d)*	$7.25^{a} \pm 0.37$	$8.76^{ab} \pm 0.39$	$9.20^{ab} \pm 0.59$	$9.72^{b} \pm 0.52$

‡T_0: Control (basal complete diet without feed additives); T_1: Complete diet incorporated with 0.60% substrate DM of EFE mix alone; T_2: Complete diet incorporated with 4.50% substrate DM of wormwood herb alone; T_3: Complete diet incorporated with 0.60% substrate DM of EFE mix and 4.50% substrate DM of wormwood herb in-combination

The means within rows with different lowercase superscripts differ significantly among the groups (*p <0.05)

DCP: digestible crude protein; DCPI : digestible crude protein intake; ME : metabolisable energy; MEI: metabolisable energy intake

was noted in T_2 at 60 (p <0.05) and 90 (p <0.01) days as compared to T_1. These results were in accordance with those of Perme and Chaudhary (2014) who also reported no effect of dietary incorporation of the herb on serum total cholesterol in buffaloes. Hypercholesterolaemic effect of dietary enzyme supplementations has also been reported by Hajati *et al*. (2009). Herbs or their active component(s) may modify fat metabolism in the body by inhibiting the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase, and/or fatty acid synthetase activity, which are the key regulatory enzymes in endogenous cholesterol synthesis (Lee *et al*., 2004).

There was significant (p <0.01) increase in HDL level at day 60 and 90 in T2 group animals, whereas significant reduction was observed in T1. The results show that addition of EFE reduces while the addition of wormwood herb increases HDL which is also evident from the significant (p <0.05) increase in HDL from day 0 to day 90 in T_2 group. There was increase in LDL level in T_0, T_1 (p <0.05) and T_3 (p >0.05) whereas decrease (p <0.05) in T_2 group from day 0 to 90 which resulted in significantly lower overall LDL values in T_2 and higher in T_1 group at day 30 (p <0.05), 60 and 90 (p <0.01). Depression in LDL was associated with an elevation in HDL levels for wormwood herb and vice-versa for EFE cocktail supplemented group, which were in accordance with the reports of Kris-Etherton and Yu (1997). Reduction in LDL is a desirable health-related goal because LDL concentrations are positively correlated with coronary atherosclerosis, while an elevation in serum HDL concentration is interpreted conversely (Amarenco *et al*., 2008). There was no change in VLDL level at day 0 and 30, whereas at day 60 significant (p <0.05) reduction was noted in T2 as compared to T_1. Likewise, at day 90, there was highly significant (p <0.01) reduction of VLDL due to supplementation of wormwood (T2) as compared to T_1. Numerical reduction in serum TC in lamb fed wormwood herb incorporated diet was primarily due to significant (p <0.01) decrease in LDL and VLDL levels. Lowest (p <0.01) AI value was observed in T_2 group at day 90 as compared to other groups; however significant (p <0.01) differences were noted among the groups at day 30 and 60 as well. Supplementation of wormwood herb lowers (p <0.05) AI values in T_2 while the values steadily increase (p <0.05) in T_0 and T_1 from day 0 to 90. These results represent the lower atherogenic potential of wormwood herb showing its lesser risk related to cardiovascular diseases, the lower the value, the lower the risk and vice versa (Lepsanovic *et al*., 2002).

From the overall results of the study, it could be concluded that incorporating EFE cocktail or wormwood herb either alone or in combination had no effect on serum total cholesterol, but LDL and AI were increased by EFE, while TG and VLDL were decreased by incorporation of herb alone which is a health desirable index. Wormwood herb could be incorporated as a feed additive with health-promoting effects in sheep when added alone.

Table 2: Serum lipid and lipoprotein profile of lambs fed diets incorporated with feed additives

Attribute	*Period (days)*	*Dietary treatments‡*			
		T0	*T1*	*T2*	*T3*
		Serum lipid profile (mg/dL)			
TC	0	83.34 ± 1.85	82.48^{A} ± 1.46	83.00 ± 0.78	83.04 ± 1.19
	30	84.37 ± 2.40	85.65^{AB} ± 2.72	83.13 ± 1.06	84.10 ± 1.80
	60	85.75 ± 2.36	89.49^{AB} ± 3.86	85.21 ± 1.15	85.05 ± 1.66
	90	88.25 ± 2.59	93.35^{B} ± 4.59	85.42 ± 0.74	86.31 ± 1.65
TG	0	30.42 ± 1.30	29.78^{A} ± 1.99	28.80 ± 1.03	31.16 ± 1.27
	30	31.12 ± 1.38	31.18^{AB} ± 1.92	28.44 ± 0.93	31.44 ± 1.15
	60*	32.52^{ab} ± 1.44	34.14^{bAB} ± 1.66	29.30^{a} ± 0.93	32.32^{ab} ± 1.16
	90**	33.20^{ab} ± 1.47	35.44^{bB} ±1.52	30.12^{a} ± 0.93	33.02^{ab} ± 1.10
		Serum lipoprotein profile			
HDL (mg/dL)	0	42.40 ± 1.41	41.40 ± 1.00	42.18A ± 1.08	41.76±1.24
	30	43.32 ± 1.56	42.02 ± 1.23	44.26^{AB} ± 1.16	42.26 ± 1.37
	60**	43.57^{ab} ± 1.78	41.58^{a} ± 1.18	47.36^{bBC} ± 1.14	43.06^{ab} ± 1.44
	90**	42.22^{a} ± 1.73	40.36^{a} ± 1.19	49.12^{bC} ± 1.23	43.90^{ab} ± 1.51
LDL (mg/dL)	0	34.86^{A} ± 0.52	35.12^{A} ± 0.84	35.06^{B} ± 0.81	35.05 ± 0.49
	30*	34.82^{abA} ± 0.87	37.39^{bA} ± 1.54	33.19^{aAB} ± 1.36	35.56^{ab} ± 0.76
	60**	35.67^{abA} ± 0.86	41.09^{bAB} ± 2.96	31.99^{aAB} ± 1.43	35.52^{ab} ± 0.64
	90**	39.39^{bcB} ± 1.22	45.90^{cB} ± 3.50	30.28^{aA} ± 1.40	35.81^{ab} ± 0.72
VLDL (mg/dL)	0	6.08 ± 0.26	5.96^{A} ± 0.40	5.76 ± 0.20	6.23 ± 0.25
	30	6.22 ± 0.28	6.24^{AB} ± 0.38	5.69 ± 0.19	6.29 ± 0.23
	60*	6.50^{ab} ± 0.29	6.83^{bAB} ± 0.33	5.86^{a} ± 0.19	6.46^{ab} ± 0.23
	90**	6.64^{ab} ± 0.29	7.09^{bB} ± 0.30	6.02^{a} ± 0.19	6.60^{ab} ± 0.22
AI	0	0.97^{A} ± 0.03	0.99^{A} ± 0.03	0.97^{C} ± 0.04	0.99 ± 0.03
	30**	0.95^{abA} ± 0.02	1.04^{bA} ± 0.03	0.88^{aBC} ± 0.04	0.99^{ab} ± 0.03
	60**	0.97^{abA} ± 0.03	1.15^{bAB} ± 0.07	0.80^{aAB} ± 0.04	0.98^{ab} ± 0.03
	90**	1.09^{bcB} ± 0.04	1.31^{cB} ± 0.07	0.74^{aA} ± 0.04	0.97^{b} ± 0.03

‡T_0: Control (basal complete diet without feed additives) ; T_1: Complete diet incorporated with 0.60% substrate DM of EFE mix alone; T_2: Complete diet incorporated with 4.50% substrate DM of wormwood herb alone; T3: Complete diet incorporated with 0.60% substrate DM of EFE mix and 4.50% substrate DM of wormwood herb in-combination

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

AI: Atherogenic index ; HDL: High-density lipoprotein ; LDL: Low-density lipoprotein ; TC: Total cholesterol ; TG: Total triglycerides; VLDL: Very low-density lipoprotein

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Announcement: SVSBT-2019

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The VII Annual Convention of the Society for Veterinary Science & Biotechnology (SVSBT) and ***National Seminar on "Biotechnological Advances for Improving Animal Health and Productivity"*** will be **organized at Navsari during 5-6 December, 2019** by the College of Veterinary Science & Animal Husbandry, Navsari Agricultural University, Navsari, Gujarat. The organizing committee of ***SVSBT-2019 invites abstracts*** of original and quality research work limited to 250 words by e-mail to svsbt2019@gmail.com latest ***by 15th November, 2019***. Details of Seminar will be available on website nau.in. ***For Further details, please contact:***

Dr. B. P. Brahmkshtri

Organizing Secretary cum Professor & Head
Department of Animal Biotechnology

College of Veterinary Science & Animal Husbandry

Navsari Agricultural University, Navsari – 396450 (Gujarat)

E-mail: svsbt2019@gmail.com; mobile +91 94273 86685