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Evaluation of native medicinal plants as feed additives in the Sheep ration

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ABSTRACT

The present study evaluated nutritional attributes of local medicinal herbs and analysed their effect as feed additives in sheep ration. Proximate and fiber analysis of all herbs- *Allium sativa, Cuminum cyminum, Emblica officinalis, Murraya koenigiii, Pimpinella anisum, Sapindus trifoliatus, Terminalia arjuna, Trigonella-foenum graecum, Zingiber officinale, Curcuma longa, Ocimum tenuiflorum* and *Azadirachta indica* and their further screening at different inclusion levels of 3%, 3.5% and 4% of ration to ascertain their effect on *in vitro* rumen fluid *p*H, dry matter digestibility and organic matter digestibility of ration was carried out during *in vitro* phase. *Pimpinella anisum* @ 3% of ration resulted in significant increase in *in vitro* dry matter digestibility and *in vitro* organic matter digestibility of ration. *In vivo* trial was conducted in which fourteen indigenous, non-descript, adult male sheep (body weight, 27.10±0.10 kg and age, 22-24 months) were randomly allotted into two groups and fed *ad lib.* wheat straw and concentrate mixture @ 25 g/$W^{0.75}$ along with *Pimpinella anisum* @ 3% of ration in treatment group. Comparable OM, CP, EE digestibility was seen in both groups, however, treatment group had significantly higher DM, total carbohydrate, CF, NDF, ADF and HC digestibility. Both groups had positive nitrogen, calcium and phosphorus balance, however, treatment group had significantly increased nitrogen balance. Rumen fermentation parameters were comparable in both groups. Haemoglobin, PCV, serum protein and albumin in treatment group were significantly higher while no significant variation was seen in serum globulin, Albumin:Globulin ratio, total cholesterol, ALT, AST, BUN, creatinine and blood glucose concentration between both groups. It can be inferred from the above data that *Pimpinella anisum* @ 3% of ration can effectively be used as a feed additive in sheep ration for improving feed intake, nutrient digestibility and utilisation.

Keywords: Feed additives, Herbs, *Pimpinella anisum*

Rapidly increasing human population is responsible for the increased demand of good quality and wholesome animal products. Their production is influenced by their genetic make-up, their surrounding environment, the quality of the feed which is being fed to the animal (VandeHaar *et al.* 2016) and the efficiency with which this feed is converted into value products. The increased animal production and their good health can be achieved by manipulating the rumen ecology responsible for the feed utilization efficiency especially by the ruminants (Santra *et al.* 2003). For this purpose, feed additives have been incorporated since long in their ration. Feed additives are non-nutritive substances and/or micro-organisms which when added to animal feed improve growth performance, feed intake and feed utilization efficiency for healthy, economic, eco-friendly and sustainable livestock production (Singh *et al.* 2015) as they are intended to increase dry matter intake, provide vital macro and micro-nutrients, improve nutrient digestibility, feed utilization, enhance growth and production performance and prevent infectious diseases occurrence in livestock.

Antibiotics have been used in sub-therapeutic doses as feed additives for improving the feed utilization efficiency, production and preventing clinical and sub-clinical infections in livestock. However, because of certain limitations like development of antibiotic resistance in the body, transfer of antibiotic residues into the food chain (Dutta *et al*. 2019, Kour *et al*. 2022), disturbance of balance among harmful and beneficial micro-organisms in the gut along with elimination of potentially beneficial micro-organisms and because of imprudent usage their use as feed additives has been discouraged. These global concerns related with the use of antibiotics as feed additive created a window of opportunities and responsibilities for animal nutritionists to search for alternative feed additives which are safer to be incorporated in the ration of animals to obtain non-residual and wholesome animal products. A wide-array of herbs exists in the pools of naturally occurring plants which are capable of positively manipulating the rumen ecology and enhancing the production performance and immune response in animals. These herbal plants are rich in secondary compounds like organic acids, essential

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oils, alkaloids, phenolic compounds (Liu *et al*. 2011) which strive their effect due to their anti-oxidant, anti-microbial, anti-inflammatory and immuno-stimulatory activity. The various outcomes expected by incorporation herbs as feed additives can be abridged as increased feed intake by the animal, increased digestibility, achievement of balance between harmful and beneficial microflora in the gut leading to better utilization of nutrients, growth and production performance (Costa *et al*. 2013, Kumar *et al*. 2014). So, there is need of illuminating various active components of herbs and the possible explanation of their mechanism of action so that they can be incorporated with much efficacy and safety after standardizing correct dosage regime in a specific formulation of feed.

MATERIALS AND METHODS

This research was carried out under the Division of Animal Nutrition, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R. S. Pura, Jammu located at 32.63° North (Latitude) and 74.73° East (Longitude). The average temperature varies from 44°C in summer to 6°C in winter. The study was conducted during the months from December-February. The average thermal oscillation during these months varied between 3°C at night and 15°C during day time.

In vitro trial

Collection and processing of herbs: Leaves of *Azadirachta indica* (Neem), *Murraya koenigii* (Curry leaves*), Ocimum tenuiflorum* (Tulsi) and bark of *Terminalia arjuna* (Arjun) were procured from their respective plants/ trees from the Horticulture department, Jammu. Other herbs-*Allium Sativa* (Garlic), *Cuminum Cyminum* (Cumin seeds), *Emblica officinalis* (Amla), *Sapindus trifoliatus* (Reetha), *Pimpinella anisum* (Sounf), *Trigonella foenum-graecum* (Methi) and *Zingiber officinale* (Ginger) were collected from the local market. Rhizome of *Curcuma longa* (Turmeric) was procured from a local farmer. Herbs were dried in the shade and further dried at 40°C for 24 h in hot-air oven followed by their grinding to 1 mm particle size.

In vitro screening: The herbs were subjected for proximate composition, fiber fractions and *in vitro* screening by incubating them with sheep rumen liquor and ration containing roughage and concentrate in the ratio of 60:40 at three different inclusion levels, i.e. 3, 3.5 and 4% of ration in order to select a particular herb at an optimum level by evaluating their effect on *in vitro* rumen fluid *p*H, IVDMD (*in vitro* Dry Matter Digestibility) and IVOMD (*in vitro* Organic Matter Digestibility) of the ration for incorporating it in the diet of the indigenous and non-descript male sheep. On the basis of screening, *Pimpinella anisum* @ 3% of ration was selected for incorporation in ration during *in vivo* study.

In vivo trial: Based on the *in vitro* results, the selected herb along with the ration was subjected to a feeding trial of 4-weeks followed by metabolism trial during the last week.

Experimental animals-housing and management: Total 14 indigenous, non-descript, adult male sheep with average body weight 27.10±0.10 kg and age 22-24 months were allotted into two groups designated-Control and Treatment group in CRD. Animals were tagged and kept in well-ventilated and hygienic pens under homogenous management conditions. They were treated with Butox® and Panacur® for ecto and endo-parasites, respectively two days before the commencement of trial.

Feeding regime and body weight record: Both control and treatment groups were given basal diet consisting of *ad lib.* wheat straw and concentrate mixture @ 25 g/kg $W^{0.75}$, however, treatment group animals were fortified with 3% *Pimpinella anisum* of ration. *Ad lib.* clean and fresh water was provided to the animals twice daily in the morning and evening. Total ration was divided into two equal parts and one part was offered at 9:00 AM while the other part was fed at 5:00 PM approximately. By subtracting feed residue from total feed offered at the end of day daily feed intake was estimated. Body weight of animals before feeding and watering was recorded using digital electronic weighing balance.

Metabolism trial and sampling: A metabolism trial was conducted to estimate daily feed intake, weekly body weight gain, nutrient digestibility, nitrogen, calcium and phosphorus balance, haematological and serological parameters. The animals were harnessed with urine and faeces collecting bags separately. The feed offered, residue left, faeces voided and urine excreted were recorded for individual animal per day. The feed and faecal samples were oven dried ground and preserved to determine proximate and fiber composition. Urine collected in urine bags was emptied in well-labelled amber coloured stoppered glass containers containing 20 ml 10% H_2SO_4. Aliquot of fresh faeces (1%) and urine (2% volume/volume) were preserved with 40% laboratory grade sulphuric acid for nitrogen estimation. A separate aliquot of 2 ml urine was pooled per day for mineral estimation which was stored at refrigeration temperature to prevent spoilage. The blood was collected using 16-guage for determining haematological and biochemical parameters. Rumen liquor was collected using Ryle's stomach tube and manual suction pump.

Statistical analysis: The results were analysed statistically by using SPSS (1996) computer package as per standard procedure of Snedecor and Cochran (1994). For *in vitro* study, data was analysed by ANOVA (Analysis of variance) and means having significant variation were ranked on the basis of Duncan's multiple range test. For *in vivo* study, data was analysed by independent sample t-test.

RESULTS AND DISCUSSION

Chemical composition of feedstuffs, medicinal herbs and in vitro degradability: The chemical composition % Dry Matter Basis (DMB) of wheat straw, concentrate mixture and herbs in presented in Table 1. The effect of

Table 1. Chemical composition of medicinal herbs

Herb	DM%	Moisture%	OM%	CP%	CF%	EE%	TA%	NFE%
Allium sativa	33.41	66.59	95.75	14.06	2.70	3.20	4.25	75.79
Cuminum cyminum	91.35	8.65	89.80	16.41	22.80	6.40	10.20	44.19
Emblica officinalis	90.24	9.76	94.50	4.69	15.20	1.50	5.50	73.11
Murraya koenigii	87.60	12.40	86.00	15.63	9.20	6.50	14.00	54.67
Pimpinella anisum	90.80	9.20	92.65	19.53	14.50	18.70	7.35	39.92
Sapindus trifoliatus	91.12	8.88	92.90	5.47	5.50	1.20	7.10	80.73
Terminalia arjuna	93.58	6.42	91.30	4.69	22.30	0.85	8.70	63.46
Trigonella foenum-graecum	94.90	5.10	95.80	23.44	5.50	7.80	4.20	59.06
Zingiber officinale	84.80	15.20	93.60	8.59	9.00	5.50	6.40	70.51
Curcuma longa	85.01	14.99	92.30	9.38	7.40	4.00	7.70	71.52
Ocimum tenuiflorum	91.31	8.69	84.50	20.31	10.70	3.90	15.50	49.59
Azadirachta indica	86.58	13.42	88.00	12.50	21.50	3.00	12.00	51.00

herbs at different inclusion levels on IVDMD and IVOMD of the ration containing roughage and concentrate mixture is presented in Supplementary Table 1 which shows that IVDMD and IVOMD of the ration containing 3% *Pimpinella anisum* (seed) showed the highest significant increase ($p<0.05$). Based on the *in vitro* results, 3% *Pimpinella anisum* (seed) of ration was selected for incorporation as feed additive in the *in vivo* trial.

The present study shows that incubation had no significant effect on rumen liquor *p*H level after 24 h which is in accordance with studies of Mir *et al*. 2010. Additionally, Sachan *et al*. 2014, also reported no effect on rumen fluid *p*H *in vitro.* Various studies reported positive effect on IVDMD of ration when incubated with medicinal (Mir *et al.* 2010, Sharma *et al*. 2012, Naseri *et al*. 2013, Sharma *et al*. 2013, Dagar *et al*. 2015, Kumar *et al.* 2016). However, in some studies, there was rather no (Chaturvedi *et al*. 2014) or decreased IVDMD of ration on incubation with herbs (Hernandez *et al*. 2017). As per this study, % IVDMD of ration containing 3% *Pimpinella anisum* showed the highest significant increase ($p<0.05$). Similarly, the highest significant increase in % IVOMD was achieved by 3% *Pimpinella anisum* which is acceptable in the light of comments of Mir *et al*. (2010), Naseri *et al*. (2013), Kumar *et al.* (2016) , Sahli *et al*. (2018), Chahaardoli *et al.* (2018) and Al-Marzooqi *et al.* (2021), who also suggested higher IVOMD of ration when incubated with herbs. Other results by Patra *et al.* (2010) showed decrement in IVOMD of the ration on its incubation with various herbs at different inclusion levels. The increase in ration IVDMD and IVOMD may be due to increased microbial count, shift in microflora type along with their enhanced fermentation activity due to presence of essential oils and activation of microbial enzymes related with nutrient degradability.

Effect on growth performance and nutrient intake: The body weight was recorded on a weekly basis and presented in Supplementary Table 2. The periodic live body weight (kg) was found to be statistically similar for both the groups ranging from 26.80±1.01 to 26.72±1.11; 27.04±1.18 to 27.20±1.10; 27.19±1.32 to 27.50±1.41; 27.40±1.08 to 27.80±1.30 and 27.40±1.15 to 27.98±1.23 on day 0, first week, second week, third week and fourth week in control and treatment group, respectively. The average DMI, CPI, DCPI and TDNI (g/day/animal), (g/animal/kg b.wt./day) and (g/animal/kg Mb.wt./day) of both groups are presented in Table 2 which shows that mean DMI and CPI did not differ significantly ($p>0.05$) between both groups, however, mean DCPI (g/day/animal) and TDNI (g/animal/day) and (g/animal/kg Mb. wt/day) was significantly higher ($p<0.05$) in treatment group.

Table 2. Effect on nutrient intake of the different group of animals during *in vivo* trial

Variable	Control	Treatment	Significance
DM intake (g/animal/day)	1284.48±8.32	1353.14±7.78	NS
DM intake (g/animal/kg b.wt./day)	46.55±1.34	48.00±1.22	NS
DM intake (g/animal/kg Mb.wt./day)	108.23±2.32	111.61±2.45	NS
CP intake (g/animal/day)	88.17±1.21	91.99±1.37	NS
CP intake (g/animal/kg b. wt/day)	3.37±0.13	3.33±0.17	NS
CP intake (g/animal/kg Mb. wt/day)	7.78±0.24	7.75±0.28	NS
DCP intake (g/animal/day)	59.06±1.04	68.46±0.89	*
DCP intake (g/animal/kg b.wt/day)	2.26±0.12	2.46±0.12	NS
DCP intake (g/animal/kg b.wt/day)	5.21±0.18	5.77±0.16	NS
TDN intake (g/animal/day)	903.25±4.75	949.56±6.10	*
TDN intake (g/animal/kg b. wt/day)	34.55±1.22	34.41±1.04	NS
TDN intake (g/animal/kg Mb. wt/day)	79.67±1.14	80.01±1.30	*

NS, Non-significant; *,Significant at 5% level.

Table 3. Effect on digestibility of nutrients in animals during *in vivo* trial

Variable	Control	Treatment	Significance
DM intake (g/animal/day)	1284.48±8.32	1353.14±7.78	NS
DM output (g/animal/day)	399.43±5.62	376.17±4.82	NS
DM digestibility (%)	67.72±5.31	71.25±1.04	*
OM intake (g/animal/day)	1204.48±12.76	1268.87±9.86	NS
OM output (g/animal/day)	344.98±14.56	318.27±11.32	*
OM Digestibility (%)	71.36±1.56	74.92±1.89	NS
CP intake (g/animal/day)	88.17±1.21	91.99±1.37	NS
CP output (g/animal/day)	29.11±1.12	23.93±1.18	NS
CP digestibility (%)	66.98±2.12	74.00±2.33	NS
EE intake (g/animal/day)	33.61±1.14	34.99±1.24	NS
EE output (g/animal/day)	9.24±0.98	8.56±1.02	NS
EE digestibility (%)	72.50±1.73	75.53±1.82	NS
Total carbohydrate intake (g/animal/day)	1135.22±5.67	1085±4.32	NS
Total carbohydrate output (g/animal/day)	419.34±7.82	303.36±5.75	NS
Total carbohydrate digestibility (%)	63.42±1.34	72.44±1.28	*
NDF intake (g/animal/day)	995.05±11.02	1023.33±16.45	NS
NDF output (g/animal/day)	307.35±12.32	269.55±14.44	NS
NDF digestibility (%)	69.11±1.32	73.66±2.45	*
CF intake (g/animal/day)	515.35±11.56	529.24±15.14	NS
CF output (g/animal/day)	131.58±8.53	113.53±9.72	NS
CF digestibility(%)	74.47±1.39	78.54±1.37	*
ADF intake (g/animal/day)	649.01±14.64	666.48±18.62	NS
ADF output (g/animal/day)	178.09±11.31	162.16±9.35	NS
ADF digestibility%	72.56±5.13	75.67±4.35	*
HC intake (g/animal/day)	346.04±11.45	356.85±14.89	NS
HC output (g/animal/day)	129.26±9.87	107.39±8.92	NS
HC digestibility (%)	70.83±3.42	74.66±2.92	*

NS, Non-significant; *, Significant at 5% level.

Effect on digestibility of nutrients: Effect of incorporating *Pimpinella anisum* on nutrient digestibility of experimental animals during metabolism trial is presented in Table 3 in which it can be seen that the per cent DM, ADF, CF, NDF, hemicellulose and total carbohydrate digestibility were significantly higher ($p<0.05$) in treatment group with respect to control group, but OM, CP and EE digestibility were statistically similar in both groups.

The average OM, CP, EE digestibility did not differ significantly ($p>0.05$) between both dietary groups**.** However, higher DM digestibility is in agreement with the study of Sallam *et al*. (2018). Total carbohydrate, CF and hemicellulose digestibility were also greater in the treatment group. Moreover, the treatment group had higher NDF and ADF which are in compliance with the study by Esfahani *et al.* 2016 and Prusty *et al.* 2019. Improved nutrients digestibility may be accredited to altered rumen ecology with increased cellulolytic bacteria count, their enhanced fermentation activity, provision of unknown factors like micro-elements for stimulation of beneficial and depression of unfavourable micro-organisms causing better nutrient digestion.

Nitrogen, Calcium and Phosphorus balance: The Nitrogen (N) balance data of the experimental sheep is presented in Table 4. The balance of nitrogen was positive between both dietary groups. Mean nitrogen intake and output as g/day/animal and nitrogen retention as per cent nitrogen absorbed were statistically similar ($p>0.05$) in both groups, on contrary, mean nitrogen balance, mean nitrogen absorbed as g/animal/day and nitrogen retention as percent nitrogen intake was significantly higher ($p<0.05$) in treatment group as compared to control group. The calcium and phosphorus balance is presented in Supplementary Table 3. The average calcium and phosphorus intake, outgo in faeces and urine and total excretion were all statistically similar ($p>0.05$) in both dietary groups. The calcium and phosphorus balance, retention of per cent intake and per cent absorbed were comparable ($p>0.05$) in both groups. The calcium and phosphorus balance (g/animal/day) was positive in both groups irrespective of the diet.

The average nitrogen intake and its total excretion was statistically similar ($p>0.0.5$) in both groups. Further, nitrogen balance, per cent nitrogen absorption and retention was significantly greater ($p<0.05$) in treatment group which may be associated with higher DCP intake, enhanced nutrient absorption and utilization. Both the groups were statistically similar with respect to average calcium and phosphorus intake, their excretion *via* faeces and urine, their balance and retention as perc ent intake and absorption indicating no negative effect on calcium and

Table 4. Effect on nitrogen balance during *in vivo* trial

Nitrogen	Control	Treatment	Significance
Intake (g/animal/day)	14.11±1.12	14.72±1.35	NS
In faeces (g/animal/day)	5.04±0.45	5.26±0.52	NS
In urine (g/animal/day)	4.47±0.67	3.78±0.52	*
Out go (g/animal/day)	9.51±1.11	9.40±1.04	NS
Nitrogen balance (g/animal/day)	4.60±0.92	5.32±0.52	*
Nitrogen absorbed (g/animal/day)	4.87±0.34	5.77±0.63	*
Nitrogen balance of % intake	31.47±2.38	34.75±3.10	*
Nitrogen balance of % absorbed	47.15±6.15	46.28±7.35	NS

NS, Non-significant; *, Significant at 5% level.

phosphorus metabolism by incorporating 3% *Pimpinella anisum* seeds in ration.

Rumen fermentation parameters: The effect of adding *Pimpinella anisum* @ 3% of ration in the experimental animals on rumen fluid *p*H, ammonia nitrogen, total nitrogen, TCA-precipitated nitrogen and non-protein nitrogen is given in Table 5. The findings revealed that there is no statistical difference ($p>0.05$) in rumen fluid *p*H, ammonia nitrogen (mg/dl), total nitrogen (mg/dl), TCA-ppt nitrogen (mg/dl) and non-protein nitrogen (mg/dl) between the two dietary groups during different dietary periods.

Incorporating 3% *Pimpinella anisum* of ration divulge no statistical difference ($p>0.05$) in rumen fluid *p*H, ammonia nitrogen, total nitrogen, TCA-ppt nitrogen and non-protein nitrogen between the two dietary groups at different dietary periods during the trial period suggesting no adverse effect on rumen fermentation profile.

Haemato-biochemical profile and serum enzymes: The effect of adding *Pimpinella anisum* on blood biochemical parameters (Table 6) showed that there was a significant increase ($p<0.05$) in hemoglobin (Hb) (g/dl), PCV (%), serum protein (g/dl) and serum albumin (g/dl) at the end of the trial in treatment group. Serum globulin (g/dl), albumin:globulin ratio, total cholesterol (mg/dl), blood glucose (mg/dl) and serum enzymes like ALT (IU/L), AST (IU/L), BUN (mg/dl) and creatinine (mg/dl) were comparable between groups at different dietary periods.

Table 5. Effect on rumen fermentation parameters during *in vivo* trial

Attribute	Control	Treatment	Significance
*p*H of rumen fluid			
0thday	6.65±0.10	6.72±0.08	NS
28thday	6.45±0.15	6.57±0.14	NS
Ammonia Nitrogen (mg/dl)			
0thday	19.54±2.72	18.24±2.45	NS
28thday	19.77±3.12	19.72±3.95	NS
Total Nitrogen (mg/dl)			
0thday	95.45±4.52	99.45±6.12	NS
28thday	101.67±6.71	99.10±6.40	NS
TCA-ppt Nitrogen (mg/dl)			
0thday	49.15±4.82	49.18±3.25	NS
28thday	50.57±4.76	49.75±3.85	NS
NPN-Nitrogen (mg/dl)			
0thday	47.72±3.42	50.12±2.22	NS
28thday	51.35±4.45	49.67±2.95	NS

NS, Non-significant; *, Significant at 5% level.

Table 6. Effect on haemato-biochemical parameters during *in vivo* trial

Blood parameter	Control	Treatment	Significance
Haemoglobin (Hb g/dl)			
0th day	7.42±0.28	7.10±0.62	NS
28th day	8.10±0.51	9.70±0.11	*
Packed cell volume (PCV %)			
0th day	25.52±1.12	22.45±2.10	NS
28th day	25.98±0.65	30.78±0.72	*
Total serum protein (g/dl)			
0th day	8.90±1.16	8.22±1.14	NS
28th day	5.64±0.72	6.79±0.81	*
Serum albumin (g/dl)			
0th day	3.82±0.68	2.20±0.79	NS
28th day	2.52±0.14	3.71±0.24	*
Serum globulin (g/dl)			
0th day	4.91±1.16	6.10±1.08	NS
28th day	3.10±0.20	2.95±0.22	NS
A:G ratio			
0th day	0.91±0.11	0.42±0.14	NS
28th day	0.84±0.08	1.32±0.11	NS
Total cholesterol (mg/dl)			
0th day	82.85±4.35	87.12±5.32	NS
28th day	84.78±5.32	85.06±4.67	NS
AST/SGOT (IU/L)			
0th day	96.15±6.35	75.10±5.42	NS
28th day	117.14±4.14	123.18±5.16	NS
ALT/SGPT (IU/L)			
0th day	7.15±1.12	12.72±1.47	NS
28th day	13.64±1.72	14.85±1.93	NS
Blood glucose (mg/dl)			
0th day	48.10±1.11	49.16±1.67	NS
28th day	57.45±1.43	59.55±1.43	NS
BUN (mg/dl)			
0th day	96.38±1.21	97.75±1.38	NS
28th day	98.55±1.54	99.32±1.78	NS
Creatinine (mg/dl)			
0th day	5.67±0.94	6.12±0.98	NS
28th day	6.10±0.78	6.89±0.87	NS

NS, Non-significant; *, Significant at 5% level.

The mean Hb level was not within normal range of 9-14 g/dl as reported by Kaneko *et al.* (2008) at the initial days of study in either of the group and on 28th day Hb was still not in normal range in control group, however, it increased significantly in treatment group which may be due to higher iron concentration of anise seeds which complies with the results of Abdelhamid *et al*. (2011). The mean PCV percent, total serum protein Abdelhamid *et al*. (2011) and serum albumin Muhamad *et al.* (2021) were significantly higher ($p<0.05$) in treatment group with respect to control group (25.98±0.65) on 28th day of feeding 3% *Pimpinella anisum*. On the contrary, Demirhan and Karafakioğlu (2022) reported no variation in serum protein and albumin level and Sharma *et al.* 2016, Bhong *et al.* 2020, Muhamad *et al.* 2021 reported variation in serum protein level.

The mean serum globulin was comparable in both groups which are in conformity with results of Muhamad *et al.* (2021). Similarly A:G ratio was statistically similar ($p>0.05$) in both groups at different dietary periods. No significant difference ($p>0.05$) was seen in total cholesterol concentration between both groups which comply with the results of Muhamad *et al.* (2021) and Demirhan and Karafakioğlu (2022). However, Sallam *et al*. (2018) and Abdelhamid *et al*. (2011) reported reduced cholesterol concentration. They observed no significant variation ($p>0.05$) in AST and ALT concentration at different dietary periods in both groups which complies with Demirhan and Karafakioğlu (2022) results, however, is in disparity with findings of Abdelhamid *et al*. (2011).

No significant difference ($p>0.05$) was seen in blood glucose concentration in both groups which is in harmony with the results by Demirhan and Karafakioğlu (2022). No significant variation ($p>0.05$) was seen in serum BUN and creatinine level between both groups at different dietary periods which is justifiable as per the observation of Muhamad *et al.,* (2021) who found no difference in BUN concentration which is similar to our result.

During *in vivo* study, incorporating *Pimpinella anisum* @ 3% of DM resulted in higher intake of DCP and TDN and higher nutrient digestibility coupled with no negative impact on rumen fermentation profile. It can be inferred that *Pimpinella anisum* @3% of DM can successfully be incorporated an as alternative natural feed additives in the ration of sheep to improve their feed utilization efficiency.

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