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Effect of probiotics mix supplementation on haemato-biochemical parameters and bacterial faecal shedding in Corriedale lambs fed paddy straw based complete feed

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ABSTRACT

Growth trial of 90 days was conducted on 12 male Corriedale lambs, divided in two groups of six lambs each, to study the effect of feeding of paddy straw based complete feed supplemented of probiotic mix (*Saccharomyces cerevisiae* 2×10^{10}cfu/g + *Lactobacillus acidophilus* 6×10^{9}cfu/g) in equal ratio @ 3 % of DM, as per the *in vitro* studies, while complete feed without probiotics served as control. There was significantly (P<0.01) higher haemoglobin concentration, RBC, WBC and monocyte count in animals fed probiotic mix than control, with no significant effect on PCV, lymphocyte, eosinophill and neutrophill count. Blood glucose, total serum protein, albumin, globulin, BUN and serum creatinine values were statistically higher in probiotic mix supplemented animals. Lambs fed with probiotics show significant (P<0.01) decrease in serum cholesterol, LDL-cholesterol and triglycerides with increment of HDL-cholesterol than animals fed unsupplemented diet, with no effect on enzyme activities of liver enzymes. The lambs fed on probiotic mix shows significant reduction in faecal shedding of *E. coli* and total coliform with firm pellets faecal consistency. Probiotic mix has great potential to beneficially affect the haemato-biochemical parameters and gut micro-flora and hence improve physiological health status of animals.

Key words: Bacterial faecal shedding, Complete feed, Haemato-biochemical, Probiotics mix.

INTRODUCTION

Probiotics are live microbial supplements when administered in adequate amounts, confer a beneficial effect on the health of the host by improving its intestinal microbial balance (FAOD WHO, 2001; Fuller, 1989). Use of microorganism (probiotics) as feed additives is widely promoted as an alternative to synthetic feed additives like antibiotics, hormones, ionophores etc owing to the risk to environment and consumers due to their residual effect. Among the numerous microorganisms, selected as probiotics in ruminants, *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* got maximum attention among animal nutritionist. Use of multi-species probiotic preparations have been reported to have synergistic effects on animal health and performance (Whitley *et al*., 2009; Doto *et al*., 2011). Apart from improving average daily gain, digestibility and rumen fermentation in growing lambs (Soren *et al*., 2013; Deka 2009) these probiotics have been reported to improve heamatological and biochemical parameters in lambs indicating good effect on physiological health status and positive nutritional status (Milewski and Sobiech, 2009; Hussein, 2014). Supplementation of diets with probiotics reported improved blood glucose (Hussein, 2014), lowered blood urea nitrogen (Bruno *et al*., 2009) and lowered Serum creatinine (Milewski and Sobiech, 2009) with normal values of liver enzymes (ELMoghazy *et al*., 2015) in lambs/sheep. These probiotics enhance immunity, maintain balance of intestinal micro-flora thus reduced the incidence of intestinal infections with positive effect in management of diarrhea (Musa *et al*., 2009). With increased health consciousness, consumers prefer diets low in cholesterol or other blood lipids such as triglycerides as it increases the risk of chronic atherosclerosis and many health problems of human including coronary heart diseases (Lim *et al*., 2004). There has been ample evidences related to control of serum cholesterol and triglyceride levels by using probiotics and has been well recognized (Kalavathy *et al*., 2006). So the present study was done to determine the effect of probiotic mix containing *Saccharomyces cerevisae* and *Lactobacillus acidophilus* on haemato-biochemical parameters and bacterial faecal shedding in Corriedale lambs fed paddy straw based complete feed.

MATERIALS AND METHODS

A growth trial of 90 days was conducted on 12 male Corriedale lambs (3-4 months old, 9.25-11.00 kg) and of uniform conformation divided in two groups of six lambs in each group, to study the effect of feeding of paddy straw based complete feed without or with supplementation of probiotic mix. A complete feed was prepared containing paddy straw 50 parts and concentrate mixture 50 parts on

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Table 1: Ingredient and chemical composition of experimental feeds.

Item	Control diet	Probiotics supplemented
Ingredients proportion (%)		
Paddy straw	50.00	50.00
Maize	6.00	6.00
Wheat bran	7.60	7.60
Deoiled rice bran	9.00	9.00
Mustard oil cake	10.00	10.00
Soyabean	15.00	15.00
Molasses	0.80	0.80
Mineral mixture	0.80	0.80
Urea	0.40	0.40
Common salt	0.40	0.40
Probiotic mix (*Saccharomyces cerevisae* + *Lactobacillus acidophilus*)	-	3.0
Chemical composition (% DM)		
CP	15.51	15.75
EE	3.15	3.18
CF	21.64	21.64
NFE	51.06	50.77
TA	8.64	8.66
AIA	3.31	3.33
NDF	68.03	67.79
ADF	42.15	42.11
HC	25.88	25.68
Cellulose	34.37	34.41
ADL	5.49	5.15
Ca	1.93	1.94
P	0.59	0.61

Note: Mineral mixture consisted of Vitamin A-7,00,000 I.U, Vitamin D3-70, 000 I.U, Vitamin E-250mg, Nicotinamide-1000mg, Co-200mg, Cu - 2000mg, I - 325mg, Fe - 1500mg, Mg - 6000mg, Mn - 1500mg, K - 100mg, Na - 5.9mg, S - 0.72%, Zn - 15gm, Ca - 25% and P - 12.75%.

DM basis to meet the nutrient requirement of animals as per ICAR (2013). The parts of concentrate mixture were maize 6.0, wheat bran 7.6, deoiled rice bran 9.0, mustard oil cake 5.0, soyabean 20.4, molasses 0.8, mineral mixture 0.8 and salt 0.4 (Table 1). Probiotic mix (*Saccharomyces cerevisiae* 2×10^{10}cfu/g + *Lactobacillus acidophilus* 6×10^{9}cfu/g) in equal ratio was incorporated in complete feed @ 3 % of DM, as per the *in vitro* studies carried to arrive at optimum level of incorporation of probiotic mix to paddy straw based complete feed for efficient utilization in ruminant system, while complete feed without probiotics served as control. Various haemato-biochemical parameters were investigated at start of the experiment and monthly intervals to judge the physiological health conditions of the experimental animals. Hb, PCV, RBC's, WBC's, lymphocytes, granulocytes, neutrophill, monocyte and eiosinophill were estimated by automatic hematology analyzer (SB-21 Vet). Glucose was estimated by glucometer instantly after blood collection. Total protein, albumin, BUN, creatinine and serum enzymes (AKP, AST, ALT and CK) were estimated by using commercial diagnostic kits (Span diagnostics limited, Surat, Gujarat, India). Globulin was determined by subtracting albumin from total protein. Serum total cholesterol, triglyceride and HDL cholesterol levels were measured using commercially available diagnostic kits (DiaSys diaganostic systems). For enumeration of *E. coli* and total coliform bacteria (CFU/g of faeces), faecal samples were collected directly from the rectum of each lamb using clean latex glove at 0, 30, 60 and 90 days of the experiment and immediately fecal isolation of *E. coli* and total coliform bacteria was performed according to Chapman and Siddons (1994).

Fecal consistency was scored at the time of sampling according to the following scales (1= Firm pellets, 2= Normal pellets, 3= Soft pellets, 4= Soft (No pellets) but not running and 5= Soft and running) Scores of 4 and 5 were considered to be diarrhea. If diarrhea was detected, the duration was also recorded. For determination of fecal pH, fecal samples were diluted 10 folds with sterile water, mixed vigorously with vortex mixer for 1 minute and pH was determined with digital pH meter. The data obtained from the experiment was processed and analyzed statistically using the Statistical Package for the Social Sciences, Base 14.0 (SPSS Software products, Marketing Department, SPSS Inc. Chicago, USA).

RESULTS AND DISCUSSION

Haemato-biochemical studies: Hemoglobin concentration, RBC, WBC and monocyte count in lambs fed probiotic mix was found significantly ($P<0.01$) higher, with no significant effect on PCV, lymphocyte, eosinophill and neutrophill count

(Table 2). Similar results were reported by Hussein (2014) and Milewski and Sobiech (2009) in lambs fed probiotics supplemented feed. Blood glucose, total serum protein, albumin, globulin and A:G ratio was statistically higher in probiotic mix supplemented animals which confirm earlier observation in sheep fed probiotics (Hussein, 2014; Khaled and Baraka, 2011; Hillal *et al.*, 2011) supplemented diets. Lambs offered unsupplemented diet recorded significantly lower BUN and Serum creatinine values than probiotics supplemented diets, probably as a result of lower intake of protein and energy intake in unsupplemented group as also reported by Masek *et al* (2008), Bruno *et al* (2009), Issakowicz *et al* (2013), Khaled and Baraka (2011) and Hillal *et al* (2011).

Experimental lambs supplemented with probiotics had significant (P<0.01) decrease in serum cholesterol, LDL-cholesterol and triglycerides with increment of HDL-cholesterol probably due to hypocholesterimia effect of probiotics (Table 3). These results were in agreement with studies conducted on sheep fed diet supplemented with yeast culture by Abu El-Ella and Kommonna, (2013) and Mousa *et al* (2012). The differences in activities of some liver enzymes (AKP, ALT, AST and CK) in serum are considered generally as indicators of some pathological changes of tissue and organ (Kaneko *et al*., 1997). In the present study plasma enzyme activities of AKP, ALT, AST and CK were not altered by probiotic mix supplementation. These results are also in line with those of ELmoghazy *et al* (2015) and El-Marakby (2003), reported no significant differences in plasma serum (AST) and (ALT) of lambs fed biologically treated rice straw compared with control ration.

Table 2: Effect of probiotics supplementation on hematological parameters (Means ± SEM) in lambs.

Particulars	Treatment groups	
	Control group	**Probiotics group**
Haemoglobin (g %)**	9.09±0.08[a]	9.43±0.13[b]
Packed cell volume (%)	32.71±0.30	33.21±0.32
RBC (M/mm^3) **	11.02±0.14[a]	11.84±0.19[b]
WBC's (M/mm^3)	11.54±0.05	11.67±0.05
Lymphocytes (M/mm^3)	6.30±0.04	6.32±0.03
Eiosinophill(M/mm^3)	0.48±0.01	0.47±0.02
Monocyte (M/mm^3)	0.06±0.04	0.07±0.03
Neutrophill(M/mm^3)	4.36±0.04	4.37±0.04

[ab]Means superscripted with different letters in a row for a particular data differ significantly from each other **(P<0.01).

Table 3: Effect of probiotics supplementation on serum biochemical parameters and enzyme profile (Means ± SEM) in lambs.

Particulars	Treatment groups	
	Control group	**Probiotics group**
Biochemical parameters		
Total serum protein (g/dl) **	6.50±0.01[a]	6.97±0.02[b]
Serum albumin (g/dl) **	3.49±0.01[a]	3.67±0.03[b]
Serum globulin (g/dl) **	3.08±0.01[a]	3.30±0.03[b]
Albumin globulin ratio	1.13±0.01	1.12±0.02
Blood glucose (mg/dl)	58.36±0.06[a]	61.14±0.19[b]
Blood urea nitrogen level (mg/dl)	14.89±0.07[a]	15.83±0.07[b]
Serum creatinine (mg/dl)	3.62±0.003[b]	3.60±0.004[a]
Cholesterol (mg/dl)	70.76±0.35[b]	64.61 ±1.09[a]
HDL (mg/dl)	41.26±0.12[a]	44.14±0.19[b]
LDL (mg/dl)	22.86±0.28[b]	20.74±0.07[a]
VLDL (mg/dl)	3.34±0.15[b]	2.70±0.88[a]
Triglycerides (mg/dl)	25.37±0.13[b]	23.83±0.13[a]
Serum enzyme profile		
AKP (IU/l)	201.64±0.99	202.26±0.35
ALT (IU/l)	18.12±0.01	18.15 ±0.02
AST (IU/l)	100.72±0.12	100.78±0.59
CK (IU/l)	140.72±0.12	140.18±0.65

[ab]Means superscripted with different letters in a row for a particular data differ significantly from each other **(P<0.01).

Faecal studies: The average faecal *E. coli*, total coliform count and pH of experiment lambs fed on probiotic mix showed significant (P<0.01) reduction as determined at 0, 30, 60 and 90 days of the experiment. This might be attributed to incomplete colonization of the gut by the probiotic mix bacteria (Table 4). Regarding faecal consistency, animals of probiotic mix supplemented group had firm pellets, whereas during the studies intermittent diarrheal condition was seen throughout the experimental period in experimental animals without probiotic supplementation. Results indicated that the number of *E. coli* and total coliform in probiotics supplemented and control group were higher on day 0 compared with day 90. Similar result was observed by Stephens *et al*. (2007) and Kawakami *et al*. (2010) in heifers

Table 4: Effect of probiotics supplementation on bacteria counts, pH and faecal consistency (Means ± SEM) in lambs.

Period (days)	Treatment groups	
	Control group	**Probiotics group**
E. coli (Log10CFU/g of faeces)		
0	5.64±0.01	5.64±0.01
30*	5.64±0.01[b]	5.56±0.03[a]
60**	5.65±0.01[b]	5.34±0.02[a]
90*	5.65±0.02[b]	5.11±0.09[a]
Coliform (log10CFU/g of faeces)		
0	6.92±0.04	6.92±0.01
30*	6.92±0.02[b]	6.88±0.01[a]
60**	6.93±0.01[b]	6.79±0.01[a]
90*	6.93±0.01[b]	6.73±0.03[a]
Faecal pH**		
0	5.98±0.01[a]	7.24±0.13[b]
30	5.98±0.01[a]	7.41±0.07[b]
60	5.99±0.01[a]	7.59±0.02[b]
90	5.99±0.02[a]	7.64±0.01[b]
Faecal consistency		
0	4.80±0.20	4.60±0.24
30*	4.80±0.20[b]	3.80±0.37[a]
60*	4.20±0.37[b]	2.60±0.51[a]
90*	3.60±0.24[b]	1.40±0.24[a]

[ab]Means superscripted with different letters in a row for a particular data differ significantly from each other *(P<0.05), **(P<0.01).

and by Ghazanfar *et al*. (2015) and Lema *et al*. (2001) in lambs who reported that feeding yeast and lactic acid bacteria lower the incidence of diarrhea by improving faecal flora. The number of coliform is higher in the animal's suffering from diarrhea, but lower in healthy animals (Signorini, 2012). Agarwal *et al* (2002) reported that feeding probiotic mix significantly decrease faecal coliform. The relationship between probiotic mix treatment and low pH conditions in the intestines has been previously reviewed (Miyazaki *et al*., 2010; Mahmmod *et al*., 2014; Bedy, 2014) and it was reported that probiotic mix bacteria have inhibitory effects on the growth and invasive function of *E. coli* and coliform bacteria due to release of carboxylic acids such as lactic acid and acetic acid which reduce gut pH.

CONCLUSION

All the haemato-biochemical parameters were well within the normal range and there was significant effect of probiotics supplementation on Hb, PCV, RBC, WBC, blood glucose, blood urea, total serum protein albumin, globulin, A:G ratio and creatinine indicated an improvement in normal physiological status of lambs, which could be attributed to the better nutrient utilization and gut health. Changes in the haematological parameters of experimental lambs were indicative of blood-supply improvement and immunity enhancement. Changes in biochemical indices suggested that the administered yeast supplement had a stimulating effect on energy metabolism and a protective effect on renal function. Probiotic mix have great potential to beneficially affect the gut micro-flora and hence improve gut and reducing mortality ratio by inhibiting pathogenic microorganisms such as *E. coli* and coliformis bacteria.

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