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Effect of feeding probiotics mix alone and in combination with fibrolytic enzymes on gut health of Corriedale lambs

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Abstract
A trial of 90 days was conducted on 24 male Corriedale lambs of uniform conformation divided in four groups of six lambs each, to study the effect of feeding probiotic mix and fibrolytic enzyme mix on gut health. A complete feed was prepared containing paddy straw 50 parts and concentrate mixture 50 parts on dry matter basis as per ICAR 2013 with supplementation of probiotics mix (*Saccharomyces cerevisiae* + *Lactobaccilus acidophillus*) @ 3g/kg DM level and exogenous fibrolytic enzyme mix @ 9 g/kg, as per the invitro studies carried to arrive at optimum level of incorporation while complete feed without supplementation served as control. The average faecal *E. coli* and total coliform count of experiment lambs of T_1 group and in T_3 group were significantly ($P<0.01$) lower as compared to T_2 and control (T_0) group. Faecal pH showed significantly ($P<0.01$) higher values in T_1 group and in T_3 group in comparison to T_0 and T_2 groups. The faecal consistency was significantly higher in T_0 group and T_2 group than T_1 group and in T_3 group.

Keywords: Corriedale lambs, Fibrolytic enzyme mixture, Gut health, Probiotic mix

Introduction
Neonatal calf diarrhoea, caused by enterotoxigenic *E. coli*, is an important cause of morbidity and mortality in newborn ruminants is the greatest single cause of death [1, 2]. The economic losses not only occur from mortality but also from other costs including diagnostics, labor, veterinary intervention and decreased number of herd replacements as well as subsequent impaired growth performance [3]. Probiotics as direct fed microbials improve gut health by significantly decreasing the number of pathogenic gut microbes responsible for calf diarrhoea [4]. Probiotics have been reported to modify the balance of intestinal microorganisms, adhere to intestinal mucosa and prevent pathogen adherence or activation, influence gut permeability, and modulate immune function [5, 6]. Early research [7] suggested that attachment to the intestinal wall was important for enterotoxin producing strains of *E. coli* to induce diarrhea. Probiotic have great potential to beneficially affect the gut microflora and hence improve gut and reducing mortality ratio by inhibiting pathogenic microorganisms such as *E. coli*, clostridium sp. and coliformis bacteria which are sensitive to the earlier culture of beneficial bacteria like lactobacillus [8]. It has also been observed that certain lactic acid bacteria showed adjuvant properties by stimulation of a specific antibody response after injection with pathogenic microorganisms. So the present study is conducted to evaluate effect of probiotic mix containing *Saccharomyces cerevisiae* and *Lactobaccilus acidophilus* along with fibrolytic enzyme mixture on gut health in Corriedale lambs.

Material and Method
A trial of 90 days was conducted on 24 male Corriedale lambs (3-4 months old, 9.25-11.00 kg) of uniform conformation divided in four groups of six lambs each, to study the effect of feeding probiotic mix and fibrolytic enzyme mix on gut health. A complete feed was prepared containing paddy straw 50 parts and concentrate mixture 50 parts on dry matter basis as per [9] with supplementation of probiotics mix (*Saccharomyces cerevisiae* + *Lactobaccilus acidophillus*) @ 3g/kg DM level and exogenous fibrolytic enzyme mix (cellulase, amylase, protease, pectinase, β-gluconase, lipase, phytase, mannase and xylanase) @ 9 g/kg, as per the invitro studies carried to arrive at optimum level of incorporation [10], while complete feed without supplementation served as control (Table 1).

Table 1: Chemical composition of experimental feeds and feed ingredients

Item	Control diet	Probiotics supplemented	Enzyme mix supplemented	Combination (Probiotics+ Enzyme mix)
Ingredients proportion (%)				
Paddy straw	50.00	50.00	50.00	50.00
Maize	6.00	6.00	6.00	6.00
Wheat bran	7.60	7.60	7.60	7.60
Deoiled rice bran	9.00	9.00	9.00	9.00
Mustard oil cake	10.00	10.00	10.00	10.00
Soyabean	15.00	15.00	15.00	15.00
Molasses	0.80	0.80	0.80	0.80
Mineral mixture	0.80	0.80	0.80	0.80
Urea	0.40	0.40	0.40	0.40
Common salt	0.40	0.40	0.40	0.40
Probiotic mix	-	0.30	-	0.30
Enzyme mix	-	-	0.90	0.90
Chemical composition (% DM)				
CP	15.51	15.75	15.74	15.77
EE	3.15	3.18	3.16	3.16
CF	21.64	21.64	21.64	21.64
NFE	49.06	48.80	48.83	48.77
TA	8.64	8.66	8.64	8.66
AIA	3.31	3.33	3.31	3.32
NDF	68.03	67.79	68.03	68.03
ADF	42.15	42.11	42.15	42.15
HC	25.88	25.68	25.88	25.88
Cellulose	34.37	34.41	34.37	34.37
ADL	5.49	5.15	5.15	5.15
Ca	1.93	1.94	1.94	1.95
P	0.59	0.61	0.60	0.61

Note: Mineral mixture consisted of Vitamin A-7,00,000 I.U, Vitamin D_3-70, 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%.

For enumeration of *E. coli* and total coliform bacteria, 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 packed in sterile plastics bags in ice and transported to microbiological laboratory. Immediately after arrival in the laboratory, fecal isolation of *E. coli* was performed according to [11]. 4 g of duplicate faecal samples were aseptically added to 36ml of 0.85% phosphate buffered saline solution (PBS) and homogenized for 1 minute. Subsequently 1 ml of the suspension was added to 9 ml of phosphate buffered saline solution and diluted to 10^{-9}. The suspension was thoroughly mixed and 0.1ml plated on EMB agar media (Hi Media laboratories, Mumbai, India) to selectively isolate *E. coli* colonies and Violet red bile agar (VRB) was used for isolation of fecal coliform. Plates were incubated at 37 °C for 24h and visually examined for metallic sheen colonies containing *E. coli* and pink colored colonies containing coliform and to determine the number of colony forming units (CFU) per gram of faeces.

Fecal consistency was scored at the time of sampling with different 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

The average faecal *E. coli* and total coliform count (Table 2, Fig I &II) of experiment lambs fed on probiotic mix alone (T_1) and in combination with enzyme mix (T_3) were significantly ($P<0.01$) lower as determined at 0, 30, 60 and 90 days of the experiment as compared to animals fed enzyme mix alone (T_2) and control (T_0). This might be because probiotic mix has great potential to beneficially affect the gut micro-flora such as *E. coli*, clostridium sp and coliformis [12, 13]. Faecal pH (Table 3, Fig III) showed significantly ($P<0.01$) higher values in probiotic mix alone (T_1) and in combination with enzyme mix (T_3) in comparison to T_0 and T_2. However, supplementation of enzyme mix alone (T_2) did not show any significant effect on pH as compared to control (T_0). The relationship between probiotic mix treatment and low pH conditions in the intestines has been previously reviewed, and some *invitro* studies have demonstrated that probiotic mix bacteria release carboxylic acids such as lactic acid and acetic acid, and have inhibitory effects on the growth and invasive function of *E. coli* in low pH conditions [14, 15].

The values of faecal consistency (Table 3, Fig IV) were significantly higher in Control (T_0) and enzyme mix alone (T_2) showing loose soft pellets than probiotic mix alone (T_1) and in combination with enzyme mix (T_3) with firm fecal pellets. During the studies intermittent diarrheal condition were seen throughout the experimental period in control group animals. These results are in accordance with reports of [4] found reduction in the incidence of diarrhoea in calves fed milk fermented with either mixed lactic acid bacteria, or *L. acidophilus 15* or *S. cerevisiae NCDC49.* Effect of period irrespective of treatments was recorded during growth trial of 90 days with values of *E. coli* and total coliform count and feacal consistency significantly ($P<0.01$) higher value at beginning of experiment and lower at end.

Table 2: Average faecal *E. coli* and Coliform shedding in different treatment groups

Period (days)	Treatment groups				
	T_0	T_1	T_2	T_3	Mean**
	***E. coli* (Log_{10} CFU/g of faeces)**				
0	5.64±0.01	$5.64±0.01^{C}$	5.64±0.01	$5.63±0.003^{C}$	$5.64±0.01^{C}$
30	$5.64±0.01^{b}$	$5.56±0.03^{aC}$	$5.65±0.01^{b}$	$5.53±0.03^{aBC}$	$5.60±0.01^{BC}$
60	$5.65±0.01^{c}$	$5.34±0.02^{aB}$	$5.65±0.01^{c}$	$5.44±0.04^{bB}$	$5.52±0.03^{B}$
90	$5.65±0.02^{b}$	$5.11±0.09^{aA}$	$5.65±0.01^{b}$	$5.20±0.08^{aA}$	$5.40±0.06^{A}$
Mean**	$5.65±0.01^{b}$	$5.42±0.05^{a}$	$5.65±0.004^{b}$	$5.45±0.04^{a}$	
	Coliform (log_{10} CFU/g of faeces)				
0	6.92±0.04	$6.92±0.01^{C}$	6.92±0.01	$6.92±0.01^{C}$	$6.92±0.02^{C}$
30	$6.92±0.02^{b}$	$6.88±0.01^{aC}$	$6.92±0.01^{b}$	$6.87±0.02^{aBC}$	$6.90±0.01^{BC}$
60	$6.93±0.01^{c}$	$6.79±0.01^{aB}$	$6.93±0.01^{c}$	$6.83±0.02^{bB}$	$6.87±0.02^{AB}$
90	$6.93±0.01^{b}$	$6.73±0.03^{aA}$	$6.93±0.01^{b}$	$6.75±0.02^{aA}$	$6.84±0.03^{A}$
Mean**	$6.93±0.002^{b}$	$6.83±0.02^{a}$	$6.93±0.002^{b}$	$6.84±0.02^{a}$	

Means superscripted with different letters in a row (^{abcd}) or column (^{ABCD}) for a particular data differ significantly from each other **

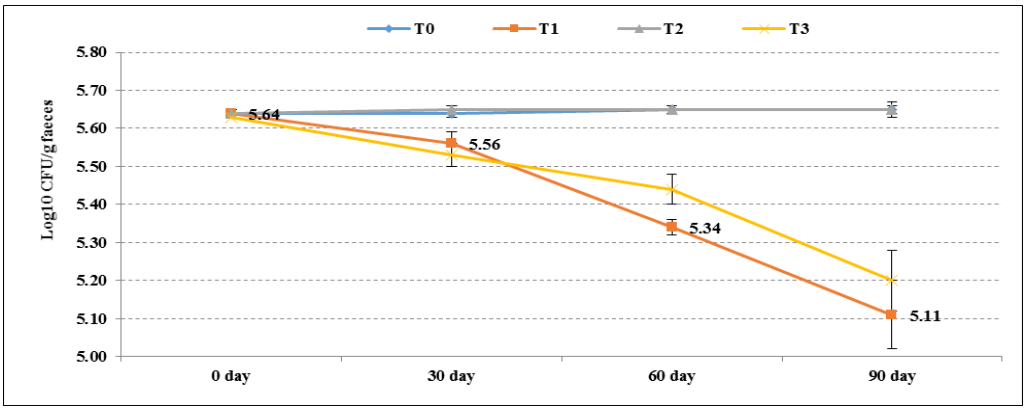

Fig I: Average faecal *E. coli* shedding in different treatment groups

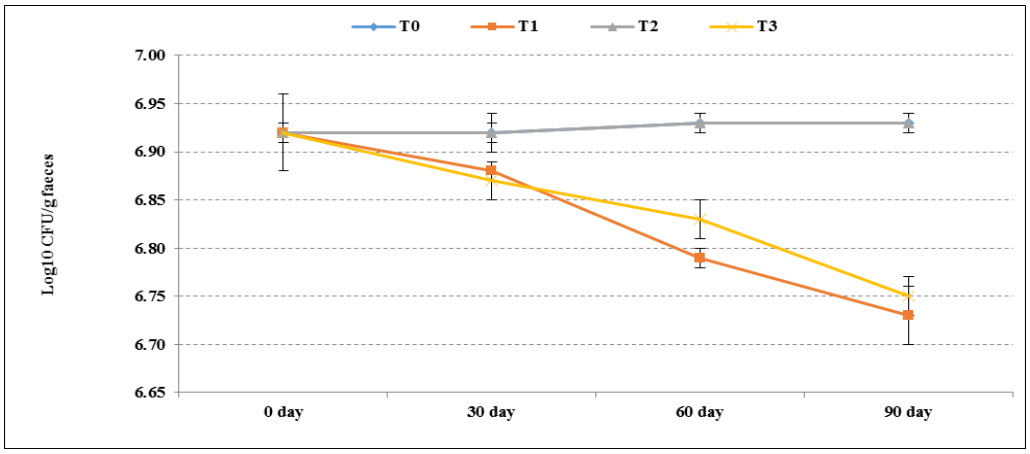

Fig II: Average faecal Coliform shedding in different treatment groups

Table 3: Average faecal pH and consistency in different treatment groups

Period (days)	Treatment groups				
	T_0	T_1	T_2	T_3	Mean**
	pH				
0	$5.98±0.01^{a}$	$7.24±0.13^{cA}$	$6.63±0.01^{bB}$	$7.25±0.11^{cA}$	6.78±0.13
30	$5.98±0.01^{a}$	$7.41±0.07^{cAB}$	$6.54±0.04^{bB}$	$7.47±0.06^{cB}$	6.85±0.14
60	$5.99±0.01^{a}$	$7.59±0.02^{cB}$	$6.47±0.06^{bB}$	$7.54±0.04^{cB}$	6.90±0.16
90	$5.99±0.02^{a}$	$7.64±0.01^{cB}$	$6.25±0.11^{bA}$	$7.63±0.01^{cB}$	6.88±0.18
Mean**	$5.99±0.004^{a}$	$7.47±0.051^{c}$	$6.47±0.044^{b}$	$7.47±0.044^{c}$	
	Faecal consistency				
0	$4.80±0.20^{B}$	$4.60±0.24^{C}$	$4.80±0.20^{B}$	$4.80±0.20^{D}$	$4.75±0.10^{C}$
30	$4.80±0.20^{bB}$	$3.80±0.37^{aC}$	$4.80±0.20^{bB}$	$3.40±0.24^{aC}$	$4.20±0.19^{C}$
60	$4.20±0.37^{bAB}$	$2.60±0.51^{aB}$	$4.00±0.32^{bA}$	$2.20±0.20^{aB}$	$3.25±0.16^{B}$
90	$3.60±0.24^{bA}$	$1.40±0.24^{aA}$	$3.60±0.24^{bA}$	$1.20±0.20^{aA}$	$2.45±0.29^{A}$
Mean**	$4.35±0.17^{b}$	$3.10±0.32^{a}$	$4.30±0.16^{b}$	$2.90±0.32^{a}$	

Means superscripted with different letters in a row (^{abcd}) or column (^{ABCD}) for a particular data differ significantly from each other **

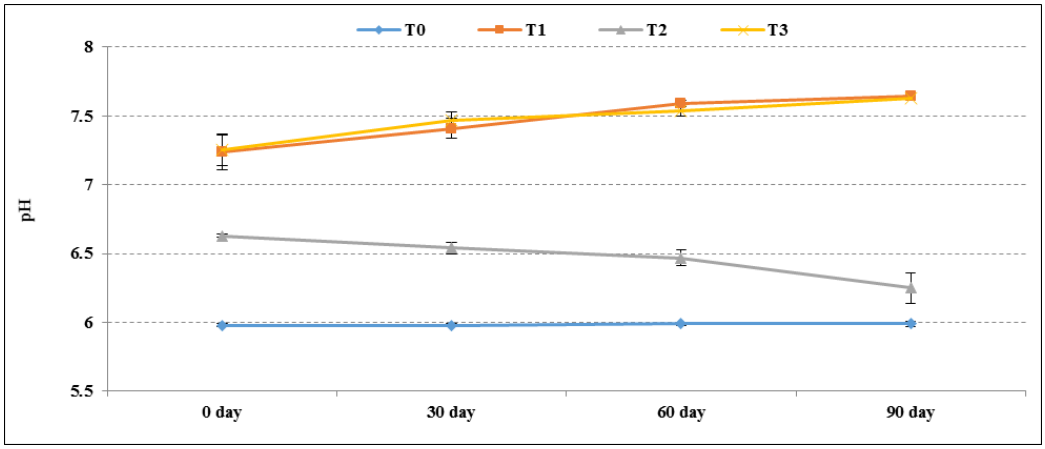

Fig III: Average faecal pH in different treatment groups

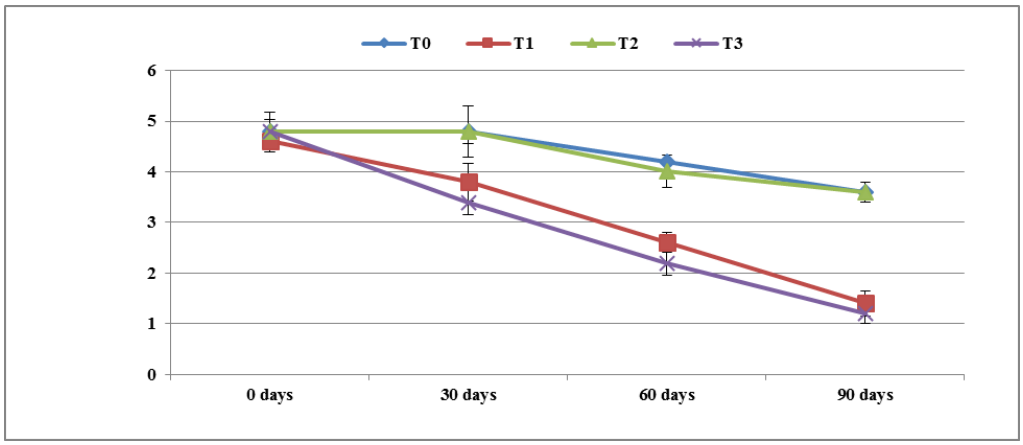

Fig IV: Average faecal consistency in different treatment groups

Conclusion

Probiotic mix alone as well as in combination with fibrolytic mix have great potential to beneficially affect the gut micro-flora and gut environment hence improve gut health by inhibiting pathogenic microorganisms such as *E. coli* and coliformis bacteria.

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