

Original Research Article

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Efficacy of Synbiotics as an Adjunct to Antibiotic Therapy in Diarrhoeic Cattle

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ABSTRACT

Diarrhoea is one of the most important problem causing great economic loss to dairy industry by drastic reduction in milk production. The present study was carried out in cattle reared at different organized dairy farms in and around Durg to evaluate the therapeutic efficacy of synbiotics along with antibiotic in diarrhoeic cattle. A total of 18 cattle were selected and divided into three groups. Group I (n=6) served as normal healthy control whereas group (n=6) II and III were diarrhoeic cattle. Group II animals were treated with ciprofloxacin @ 5 mg/kg orally twice daily for 5 days and Group III were administered with combination of ciprofloxacin@ 5 mg/kg orally BID for 5 days and symbiotic (Ecotas[®]) two boli orally OD for 5 days. *Escherichia coli* (66.11%) was found to be most common pathogen involved in cattle diarrhoea followed by *Salmonella* spp. (22.22%) and *Proteus* spp. (16.66%) respectively. The hemato-biochemical alterations revealed significant increase in haemoglobin, PCV, TEC, TLC, neutrophils, total protein, albumin, albumin: globulin ratio and potassium values whereas, there was a significant decrease in lymphocyte, monocyte, eosinophil, globulin, sodium and glucose levels which returned near to normal values over the due course of treatment period in Group III followed by Group II. Rumen function profile revealed significant decrease in rumen pH, sedimentation activity time and protozoan motility values, while cellulose digestion and methylene blue reduction values were increased in diarrhoeic cattle. The rumen function profile returned to normal in Group III followed by Group II. Hence, combination of antibiotic along with symbiotic proved to be a better alternative when compared to antibiotic alone for therapeutic management of diarrhoea in cattle.

Keywords

Synbiotics,
Antibiotic therapy,
Diarrhoeic cattle,
Hemato-
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Introduction

Gastrointestinal infections are one of the most common illness caused by different groups of microorganisms and these are leading cause

of death in India. Among GIT infections diarrhoea is commonly reported which is defined as an increased frequency, fluidity or volume of faecal excretion. In diarrhoea, the clinico-biochemical alterations are complex in

nature characterized by imbalance of fluid, electrolyte and acid-base status (Radostits *et al.*, 2010). Maintaining a healthy gastrointestinal microbiota is critical for the health and productivity of ruminants. However, antibiotic administration in cattle is widely used for the treatment of gastrointestinal infections. Antibiotic therapy may disturb the indigenous microbiota as well as increased antibiotic-resistant genes in dairy cows (Liu *et al.*, 2016). Antibiotics suppress the rumen and intestinal microflora which ultimately leads to production losses and prolonged the recovery (Ravi *et al.*, 2015). Now a days, the emergence of antibiotic resistance in the human commensal bacteria has raised concerns about the impact of antimicrobial compounds for agriculture use and has accelerated the search for alternative nutritional strategies, such as the addition of probiotics and prebiotics (Verstegen and Williams, 2002). Considering the above issues, the present work was designed to compare the efficacy of synbiotic along with antibiotic therapy.

Materials and Methods

The present study was carried out in cattle reared in different organized dairy farms, in and around Durg district of Chhattisgarh. Cattle showing watery diarrhoea were included. Gross examination of faeces was done to study the following parameters: fluidity of faeces, colour of faeces and presence of mucous and blood. Therapeutic management with antibiotic was based on the antibiotic sensitivity test of rectal swab against commonly used antibiotic discs (Hi Media, Limited, Mumbai) (Manickam and Ponnusamy, 2017). Two different treatment strategies were used for the therapeutic management of cattle affected with diarrhoea and the therapeutic efficacy of the drugs were assessed on the basis of faecal examination along with restoration of haemato-biochemical and rumen function profile.

Group II animals were treated with ciprofloxacin @ 5 mg/kg orally twice daily for 5 days and Group III were administered with combination of ciprofloxacin @ 5 mg/kg orally BID for 5 days and symbiotic (Ecotas[®]) two boli orally OD for 5 days.

Haematol-biochemical parameters

About 5ml blood was collected aseptically from jugular vein of all diarrhoeic and healthy cattle in EDTA coated vial using 16 gauge needle on day 0 (pre-treatment) and day 5 (post-treatment) for haemato-biochemical studies.

Haematological parameters like haemoglobin (gm/dl), TEC (millions per cubic millimeter ($10^6/\text{mm}^3$), PCV (%), TLC (thousands per cubic millimeter ($10^3/\text{mm}^3$) and DLC (%) were estimated by Vet MS39 semiautomatic haematological analyzer of MeletSchloesing Laboratories of Sussi France.

Serum samples were harvested from blood from diarrhoeic and healthy cattle in serum vials on day 0 (pre-treatment) and day 5 (post treatment). Biochemical parameters like glucose (mg/dl), total protein (g/dl), albumin (g/dl), globulin (g/dl), albumin-globulin ratio, aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L) were estimated by DiaSIL-100 semiautomated Biochemistry Analyzer (Systronics India Limited), as per the standard method and procedures given in commercial kits supplied by ARKRAY Healthcare Pvt. Ltd. Serum sodium (mmol/l) and potassium (mmol/l) were estimated by using microprocessor based flamephotometer Model- 1382/1385 (ESICO).

Rumen fluid examination

Rumen fluid samples from all diarrhoeic and healthy cattle were collected by needle puncture of the rumen (rumenocentesis) from

left lower paralumbar fossa (Petrovski, 2017). The following parameters were evaluated on day 0 and 5 of the study:

A. Physical parameters: Colour, odour, consistency and Sedimentation Activity Test (SAT).

B. Chemical parameters: pH and Cellulose Digestion Test (CDT)

C. Microbial parameters: Rumen bacteria (gram staining and methylene blue reduction test), rumen protozoa by microscopic examination and protozoan motility

Statistical analysis

The results obtained were tabulated and subjected to statistical analysis by Completely Randomized Design (C.R.D.) as per the procedure outlined by Snedecor and Cochran (1994) by using IBM SPSS statistics.

Results and Discussion

Fecal examination

Examination of feces revealed that the faecal discharge in all diarrhoeic cattle varied in consistency from watery to loose with mucous and yellowish white in colour as compared to green or brownish olive solid faeces in animals of healthy control group (Group I) on day 0. However, on day 5 following treatment, fecal examination of animals of all treated groups revealed green or brownish olive solid faeces without mucous. In the present study, faecal examination of diarrhoeic revealed that the faecal discharge varied in consistency loose to watery with mucous and yellowish white in colour. The observations are in accordance with the findings of Shekhar *et al.*, (2017). Yellowish white diarrhoeic faeces might be due to high content of salt particularly bicarbonate (Ward,

1976) with heavy secretion of water in intestinal lumen (Szancer, 1980). The findings of faecal examination revealed maximum improvement of different parameters in the animals of group III, followed by group II respectively. *Escherichia coli* (66.11%) was considered to be most common pathogen involved in cattle diarrhoea followed by *Salmonella* spp. (22.22%) and *Proteus* spp. (16.66%) respectively.

Haematological parameters

Haemoglobin (g/dl)

The changes in haemoglobin, PCV, TEC, TLC and WBC were studied on 0th and 5th day (Table 1).

The present study elucidated a significant ($P<0.01$) increase in haemoglobin concentration in diarrhoeic animals as compared to the healthy control group. Restoration of haemoglobin value was recorded in treatment groups. The findings of increased haemoglobin values on the day 0 in the present study are in corroboration with the findings of Shekhar *et al.*, (2017). The mean haemoglobin values in diarrhoeic cattle were found to be increased, which might be due to haemoconcentration because of dehydration and drainage of a large quantum of body fluids in watery purgation. However, in contrast to our findings, non-significant changes in haemoglobin values have been observed by Singh *et al.*, (2014). However, Jain *et al.*, (2016) reported that the Hb concentration was significantly ($P<0.05$) decreased in the clinical cases of bovine diarrhoea.

A highly significant ($p<0.01$) decrease in PCV values was recorded in the animals of group II and III on day 5 following treatment as compared to day 0. The findings of present study are in accordance with those reported

by Roy *et al.*, (2009) and Shekhar *et al.*, (2017) who have also reported that increase in PCV values in diarrhoeic cattle might be due to haemoconcentration associated with dehydration and hypovolemia.

Significantly ($p < 0.05$) higher TEC values were recorded in animals of group III followed by group II respectively on day 5 following treatment. The mean TEC values toward the end of study period were recorded to be within normal range.

The findings of the present study are accordance with Shekhar *et al.*, (2017) who have also reported that mean TEC values in diarrhoeic cattle were found to be increased, which might be due to loss of extracellular fluid in diarrhoea which leads to haemoconcentration and hypovolemia.

The result of present study revealed a significant ($P < 0.01$) increase in TLC values in the animals of all groups with diarrhoea on the pre-treatment day as compare to the healthy control group. The post therapeutic TLC value decreased on day 5 following treatment in all treated groups but maximum restoration was observed in Group III which was treated with ciprofloxacin along with ecotas. The findings of increased TLC values on the day 0 in the present study are in corroboration with the findings of Shekhar *et al.*, (2017). Leukocytosis might have occurred due to normal reaction of body defense mechanism against infection and also due to dehydration and haemoconcentration. However, the reduction in TLC values following treatment might be due to control of infection in the patients during our study period. The combination of potent antibacterial agent and synbiotic might have blocked the rapid multiplication of pathogenic bacteria in the present therapeutic trial (Sharma *et al.*, 2016).

A non-significant ($p > 0.05$) decrease in neutrophil percentage was recorded in animals of group I (Healthy control) on day 5 as compared to day 0 of treatment. However, a highly significant ($p < 0.01$) decrease in neutrophil percentage values were recorded in the animals of group II and III on day 5 following treatment as compared to day 0. At the end of observation period (day 0), lymphocyte and neutrophil percentage was recorded to be well within normal physiological values.

The eosinophil percentage in all animals after treatment decreased significantly ($p < 0.001$). However, maximum decrease was observed in the animals of group III and II respectively following treatment. Percentage of eosinophil was recorded to be well within normal physiological values after treatment. Eosinopenia might have occurred due to active infectious process (Smith, 1996). The findings of normal eosinophil count in the present study corroborates with the findings of Jain *et al.*, (2016) wherein they have reported non-significant changes in eosinophil values in bovine suffering from diarrhoea.

The mean basophil percentage values differed non-significantly ($p > 0.05$) in animals of all treatment groups at different intervals as compared to that of healthy control group on day 0 and day 5. However, the mean values of healthy control group remained within the range on day 5 (post-treatment). The findings of non-significant changes in basophil values in our study are in concordance with the findings of Asati *et al.*, (2008) wherein they have also reported non-significant changes in basophil percentage in cattle suffering from diarrhoea.

The lymphocyte percentage increased significantly ($p < 0.001$) in the animals of group III and II respectively following treatment. At the end of observation period

(day 5), lymphocyte percentage was recorded to be well within normal physiological values.

The monocyte percentage of all the treated groups decreased significantly ($p < 0.01$) as the treatment proceeded. However, maximum decrease in monocyte percentage was observed in the animals of group III and II respectively following treatment which returned near to normal values on day 5. Monocytopenia might be occurred due to endotoxins (Smith, 1996). The findings of normal monocyte count in the present study corroborates with the findings of Jain *et al.*, (2016) wherein they have reported significant changes in monocyte values in bovine suffering from diarrhoea. However, Asatiet *et al.*, (2008) reported non-significant changes in monocyte values in diarrhoeic cattle. Shekhar *et al.*, (2017) have also reported significant increase in monocyte count in diarrhoeic calves.

The marked neutrophilic response with lymphopenia is characteristic of acute bacterial enteritis (Malik *et al.*, 2013) and the findings of reduced lymphocyte count in the present study might be attributed to the fact that neutrophilia leads to concurrent lymphopenia (Schalmet *et al.*, 1975).

Biochemical parameters

Mean \pm SE of glucose, total protein, albumin, globulin, A:G ratio, AST, ALT, serum sodium and potassium at various intervals of treatment (0th day and 5th day) (Table 2).

A non-significant ($p > 0.05$) increase in blood glucose value was recorded in animals of group I (Healthy control) on day 5 as compared to day 0. However, a highly significant ($p < 0.001$) increase in blood glucose values was recorded in the animals of group II and III on day 5 following treatment as compared today 0. Hypoglycaemia might

have occurred as a result of reduced rate of conversion of lactic acid to glucose (Morris *et al.*, 1985). Other factors involved in causing hypoglycaemia in diarrhoeic cattle might include anorexia, decreased intestinal absorption of glucose, alteration in tissue metabolism caused by decreased blood flow and oxygenation associated with hypovolemic shock (Bywater, 1977). The findings of decreased glucose values on the day 0 in the present study are in corroboration with the findings of Shekhar *et al.*, (2017).

The noticeable increase in the level of total serum protein (TSP) values in diarrhoeic cattle as compared to healthy control animals is in close agreement with Singh *et al.*, (2014). Earlier investigators (Shekhar *et al.*, 2017) have also reported increase in serum total protein concentration in clinical cases of cattle diarrhoea. The marked increases in total serum protein in diarrhoeic cattle might be observed apparently due to associated dehydration. Walker *et al.*, (1998) observed the significant increase in both PCV and TSP, which indicates hypovolemia, hemo-concentration and reduced glomerular filtration rate.

The noticeable increase in the level of albumin in diarrhoeic cattle as compared to healthy control animals is in close agreement with Singh *et al.*, (2014). A significant increase in serum albumin concentration in the diarrhoeic cattle has also been reported by the earlier investigators (Shekhar *et al.*, 2017). The apparent hyperalbuminemia observed in the study might be due to definitive bioresponse to abnormal loss of body fluids.

A non-significant ($p > 0.05$) increase in globulin value was recorded in animals of group I (Healthy control) on day 5 as compared to day 0 of observation period. However, a highly significant ($p < 0.01$) increase in globulin values was recorded in

the animals of group II and III on day 5 following treatment as compared to day 0. The globulin values of all the treated groups increased significantly ($p < 0.01$) after initiation of therapy. However, maximum increase was observed in the animals of group III followed by group II respectively.

The A:G ratio values of all the treated groups decreased significantly ($p < 0.01$) after treatment. The findings of increased A:G ratio values on the day 0 of treatment in the present study are in corroboration with the findings of Singh *et al.*, (2014). A significant increase in A: G ratio has also been observed in diarrhoeic cattle which might be due to either increase in albumin or due to dehydration (Kaneko, 2008), or decrease in globulin. In contrast to our findings, decreased A:G ratio in diarrhoeic cattle has been reported by Rekha *et al.*, (2011).

The mean AST values differed non-significantly ($p > 0.05$) in animals of all treatment groups at different intervals as compared to that of healthy control group on day 0 and day 5 of treatment. However, the mean values of healthy control group remained within the range on day 5 (post-treatment). Similar type of findings has also been reported by Singh *et al.*, (2014). Non-significant changes in AST activities were observed in diarrhoeic calves which indicate the absence of marked hepatic damage (Lewis *et al.*, 1975). In contrast to our findings, significant increase in the activities of AST in diarrhoeic calves as compared to normal ones has been reported by Galbat *et al.*, (2015).

A non-significantly ($p > 0.05$) differed mean ALT values were recorded in animals of all treatment groups at different intervals as compared to that of healthy control group on day 0 and day 5 of treatment. However, the mean values of healthy control group remained within the range on day 5 (post-

treatment). Similar type of findings has also been reported by Shekhar *et al.*, (2017). Non-significant changes in ALT activity indicate the absence of marked hepatic damage (Lewis *et al.*, 1975). In contrast to our findings, Galbat *et al.*, (2015) reported a significant increase in the ALT activities diarrhoeic calves.

The serum sodium values of all the treated groups increased significantly ($p < 0.01$) after initiation of therapy. However, maximum increase was observed in the animals of group III followed by group II respectively. Hyponatremia in diarrhoeic cattle might have occurred due to excessive secretion of the sodium ions by intestinal villus which is lost through the intestinal tract particularly in enterotoxigenic *E. coli* induced diarrhoea (Radostitset *et al.*, 2010).

Most of the diarrhoea causing microorganisms disrupt the intestinal function and dehydrate the body either by increasing the chloride-secreting activity of the crypt cell or impairing the absorption of sodium by the villus cells or both. Hence, the fluid that is normally returned to the blood across the intestinal wall is lost in the watery stool (Hirschhorn and Greenough III, 1991).

The serum potassium values of all the treated groups decreased significantly ($p < 0.01$) after treatment. However, maximum decrease was observed in the animals of group III and II respectively.

Hyperkalaemia might be due to increased potassium retention by kidney and also due to consequence of accelerated leakage of intracellular potassium across the structurally altered plasma membranes of damaged cells into the fluid compartment of circulating blood (Fisher and Dela Fuente, 1971) or it may be due to metabolic acidosis which might have induced translocation of potassium ions

from intracellular to extracellular compartment (Shrivastava *et al.*, 2001).

Rumen liquor examination

The changes in physical parameters, chemical parameters and microbiological parameters of rumen fluid were studied and their levels at various intervals of treatment (0 and 5th day).

Evaluation of rumen fluid revealed that the colour of rumen fluid in animals of group I and II varied from brown to light brown in colour as compared to yellowish brown or brownish green colour in the animals of group I (Healthy control) on day 0 of study. However, on day 5 following treatment, the colour of rumen fluids was yellowish brown or brownish green in animals of group I and III as compared to light brown colour in animals of group II.

Evaluation of rumen fluids revealed that odour of rumen fluid in the animals of group II and III was acidic as compared to aromatic odour of rumen fluid in the animals of group I (Healthy control) on day 0. However, on day 5 following treatment the odour of rumen fluid in the animals of group I and III was aromatic as compared to acidic odour in animals of group II.

Evaluation of rumen fluids revealed that the consistency of rumen fluids in the animals of group II and III was watery as compared to slightly viscous consistency in the animals of group I (Healthy control) on day 0.

However, on day 5 following treatment the consistency of rumen fluid was slightly viscous in the animals of group I and III as compared to watery consistency in group II. The watery consistency characteristics might be due to the presence of inactive bacteria and protozoa (Arsat *et al.*, 2015).

Sedimentation activity test (SAT in minutes)

Mean \pm SE of SAT (minutes) on day 0 in group I, II and III were 7.17 ± 0.31 , 2.33 ± 0.21 , 2.25 ± 0.25 , respectively. Similarly the SAT (minutes) values in group I, II and III on day 5 were 7.50 ± 0.22 , 4.50 ± 0.22 , 8.33 ± 0.25 respectively. The SAT values of all the treated groups increased significantly ($p < 0.01$) after initiation of therapy. However, maximum increase was observed in the animals of group III followed by group II respectively. The results of the present study with very rapid sedimentation to no floatation might be related to the prolonged anorexia, rumen acidosis and inactive ruminal microflora (Smith, 1996).

Chemical parameters

Rumen pH

A non-significant ($p > 0.05$) decrease in rumen pH value was recorded in animals of group I (Healthy control) and group II on day 5 as compared to day 0. However, a highly significant ($p < 0.01$) increase in SAT values were recorded in the animals of group III on day 5 following treatment as compared to day 0. Presumably, in the present therapeutic trial the restoration of rumen pH in animals treated with synbiotics might be due to beneficial effect of synbiotics on the balance of rumen microbiota and thereby the stabilization of pH value (Habeeb, 2017).

Cellulose digestion test

Maximum decrease in CDT values was observed in the animals of group III followed by group II post-treatment. In the present therapeutic trial, the decrease in CDT values in animals treated with synbiotics might be due to beneficial effect of synbiotics which stimulate total and cellulolytic bacterial numbers in rumen fluid (Shakira *et al.*, 2018).

Table.1 Haematological changes in healthy and diarrhoeic animals on day 0 and 5

Parameters	Groups	Treatment intervals (Days)	
		0	5
Haemoglobin (g/dl)	I	11.09 ^b ±0.20	11.30 ^{ab} ±0.19
	II	13.40 ^a ±0.18	11.62 ^{a**} ±0.06
	III	12.94 ^a ±0.13	11.15 ^{ab**} ±0.17
TEC(10 ⁶ /mm ³)	I	7.37 ^c ±0.04	7.59 ^a ±0.14
	II	9.09 ^a ±0.15	6.97 ^{b**} ±0.15
	III	8.72 ^b ±0.06	7.47 ^{a**} ±0.02
PCV (%)	I	35.47 ^C ±0.63	36.18 ^{ab} ±0.22
	II	42.95 ^a ±0.19	37.10 ^{a**} ±0.20
	III	40.77 ^b ±0.29	35.82 ^{b**} ±0.50
TLC(10 ³ /mm ³)	I	9.89 ^c ±0.12	9.73 ^a ±0.22
	II	13.78 ^a ±0.16	10.04 ^{a**} ±0.23
	III	13.61 ^a ±0.20	8.39 ^{b**} ±0.02
DLC Neutrophils (%)	I	29.80 ^b ±0.16	29.62 ^b ±0.12
	II	57.28 ^a ±0.12	30.87 ^{a**} ±0.28
	III	56.85 ^a ±0.21	29.65 ^{b**} ±0.18
Eosinophils (%)	I	2.23 ^a ±0.03	2.25±0.02
	II	1.47 ^b ±0.08	2.33 ^{**} ±0.04
	III	1.28 ^b ±0.09	2.30 ^{**} ±0.04
Basophils (%)	I	0.48±0.07	0.50±0.05
	II	0.40±0.07	0.47±0.10
	III	0.28±0.11	0.48±0.09
Lymphocytes (%)	I	64.20 ^a ±0.12	64.32 ^a ±0.21
	II	38.35 ^c ±0.18	63.07 ^{b**} ±0.23
	III	39.02 ^b ±0.33	64.33 ^{a**} ±0.12
Monocytes (%)	I	3.28 ^a ±0.04	3.32±0.04
	II	2.50 ^b ±0.07	3.27 ^{**} ±0.03
	III	2.58 ^b ±0.07	3.35 ^{**} ±0.03

Table.2 Biochemical changes in healthy and diarrhoeic animals on day 0 and 5

Parameters	Groups	Treatment intervals (Days)	
		0	5
Blood glucose (mg/dl)	I	65.40 ^a ±0.60	65.59 ^b ±0.55
	II	36.40 ±0.37	61.74 ^{d**} ±0.20
	III	36.44 ^b ±0.62	66.88 ^{a**} ±0.28
Total protein(g/dl)	I	6.94 ^b ±0.14	6.94±0.14
	II	7.71 ^a ±0.04	6.86 ^{**} ±0.04
	III	7.81 ^a ±0.06	7.04 ^{**} ±0.03
Albumin (g/dl)	I	3.60 ^b ±0.07	3.62 ±0.07
	II	4.79 ^a ±0.05	3.62 ^{**} ±0.08
	III	4.87 ^a ±0.05	3.78 ^{**} ±0.03
Globulin (g/dl)	I	3.34 ^a ±0.07	3.32±0.08
	II	2.89 ^b ±0.02	3.23 ^{**} ±0.06
	III	2.96 ^b ±0.01	3.26 ^{**} ±0.03
A:G ratio	I	1.08 ^b ±0.02	1.09±0.02
	II	1.57 ^a ±0.07	1.13 ^{**} ±0.05
	III	1.66 ^a ±0.02	1.16 ^{**} ±0.02
AST (U/L)	I	16.59±0.18	16.90 ±0.23
	II	16.46±0.18	16.59±0.09
	III	16.53±0.15	16.79±0.18
ALT (U/L)	I	37.18±0.23	37.23±0.18
	II	37.25±0.20	37.19±0.22
	III	37.49±0.19	37.61±0.13
Serum sodium (mmol/l)	I	146.00 ^a ±0.21	146.06 ^b ±0.22
	II	125.85 ^c ±0.40	142.13 ^{d**} ±0.24
	III	127.63 ^b ±0.35	147.23 ^{a**} ±0.28
Serum potassium (mmol/l)	I	4.74 ^c ±0.13	4.82 ^a ±0.11
	II	6.32 ^{ab} ±0.17	4.98 ^{a**} ±0.05
	III	6.78 ^a ±0.34	4.83 ^{a**} ±0.11

Rumen bacteria

Gram staining

Gram's staining of the rumen fluid revealed that number of Gram positive bacteria were increased in the animals of group II and III as compared to predominantly Gram negative populated rumen fluid in the animals of group I (Healthy control) on day 0. However, on day 5 following treatment the numbers of Gram negative bacteria population were predominant in rumen fluid in animals of group I and III as compared to group II.

The result of present study indicated that population of gram negative bacteria were reduced in rumen fluid of diarrhoeic cattle on the day of presentation (day 0). However, animals treated with antibiotic alone (group II) showed low population of gram negative bacteria in rumen fluid on day 5 following treatment.

Presumably, in the present therapeutic trial the increase in rumen microflora in animals treated with synbiotics might be due to a synergistic effect in the stimulation of gut microbiota (Shakira *et al.*, 2018).

Methylene blue reduction test (MBRT, minutes)

A highly significant ($p < 0.01$) decrease in MBRT values was recorded in the animals of group III on day 5 following treatment as compared to group II. The delayed clearing of the dye might be due to indicated diminished anaerobic bacteria activity (Fubini and Ducharme, 2004). Presumably, in the present therapeutic trial, the decrease in MBRT in animals treated with synbiotics might be due to a synergistic effect of symbiotic for stimulation of gut microflora (Shakira *et al.*, 2018).

Rumen protozoa

Microscopic examination of protozoa

The protozoan motility in animals of group III increased as the treatment proceeded. A non-significant decrease in protozoan motility values in animals of group II was recorded in treated animals. The findings in the present study are in corroboration with the findings of by Petrovaski *et al.*, (2017). The lower motility and reduction in number of protozoa were responsible for reduced grades of motility and protozoal activity observed and indicated that there was a disturbance in the ruminal environment.

The sluggish motility and low number of protozoa observed in the present study also might be attributed to factors like induced sub-acute rumen acidosis, fall in the pH of rumen contents and anorexia which could cause undesirable changes in microbial population (Arsat *et al.*, 2015).

Presumably, in the present therapeutic trial, the vigorous protozoan motility and optimum concentration per field in animals treated with synbiotics might be due to the effect that certain yeast are particularly effective at raising and stabilizing ruminal pH by stimulating certain populations of ciliate protozoa, which rapidly engulf starch and, thus, effectively compete with amylolytic lactate producing bacteria (Shakira *et al.*, 2018).

In conclusion, antibiotics alter the gut microflora composition and decrease its richness and diversity. However, synbiotics improve the ruminal and intestinal microbial balance. Hence, synbiotics are effective in raising and stabilizing ruminal pH by stimulating certain populations of ciliate protozoa.

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