Nigerian J. Anim. Sci. 2017 (2):247 - 260

Influence of Probiotics on Rumen Liquor Characteristics and Microbiology

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Target Audience: Ruminant Nutritionists, Goat Farmers

Abstract

The growing concerns attributed to indiscriminate usage of antibiotics has necessitated the search for alternatives. Probiotics has been noted to work synergistically with rumen microbes and improved rumen liquor characteristics. In this study, we investigated the effect of probiotics inclusion on rumen liquor characteristics (physical, chemical and fermentative qualities) and microbiology in WAD goats. In a completely randomised design, eighteen goats were allotted to six dietary treatments: control (D1); antibiotic (D2); 2.5g bakers yeast (D3); 5.0g bakers yeast (D4); 2.5g yeast plus Lactobacilli (D5) and 5.0g yeast plus Lactobacilli (D6), where D5 and D6 were fortified with Lactobacillus acidophilus at $1.00x10^{12}$cfu/g each. Rumen liquor was assessed on its colour which was generally brownish green and pH ranged from 6.70 to 6.9. Methyl blue reduction time was highest for D2 (4.83 mins) and the least was observed in D3 and D5 (4.00 mins). Fluid chloride was highest in D3 (49.13 mEq/L) and least was recorded for D6 (34.00 mEq/L). Animals on D6 (62.22 mM) recorded the highest total volatile fatty acids while those on D2 (49.67 mM) had the least. The mixed probiotic (D5 and D6: 7.85 and 8.15 mg/dl) elicited a higher ammonia nitrogen levels that was similar ($p > 0.05$) with D2 (7.33 mg/dl) but different ($p < 0.05$) from D1, D4 and D3 (5.91, 4.70 and 4.45 mg/dl). Bacteria count was highest in animals on D5 (233.33×10^6 cfu/mL) and least was seen in those on D2 (129.33×10^6 cfu/mL) while fungi population in animals on D4 (54.00×10^3 cfu/mL) recorded the highest and those on D2 had the least (26.00×10^3 cfu/mL). It was concluded that, fortification of WAD bucks diet with 2.50 g and 5.00 g of yeast and Lactobacilli improved rumen liquor characteristics and microbiology.

Keywords: Bucks, Bakers yeast, *Lactobacillus acidophilus*, rumen liquor, microbiology

Description of Problem

For the past few decades, a number of chemical feed additives such as antibiotics, ionophores, methane inhibitors and defaunating agents have been used in ruminant nutrition to manipulate the microbial ecosystem and fermentation characteristics in the rumen and intestinal tract of livestock [1]. Due to probable toxicity problems to

the host animals, these feed additives are not routinely used. Recently, a great awareness from public health aspects such as residues of these chemicals in milk and meat, and bacterial resistance to antibiotics as a result of increased use in the food chains prohibits their use as feed additives [2]. These supplements have been criticized by the consumers' organizations on the ground of product safety and quality. The consumers' demands have stimulated the search for natural alternatives to chemical feed additives. One of such effort in recent years is supplementation of Probiotics to rations of livestock since it presents an attractive alternative to the use of chemical and hormonal promoters. They are known to improve the utilization of cellulosic materials, health, productivity and reproduction [3].

The term probiotic has been defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance" [4]. There are many different types of probiotics being used in livestock production. They can be classified into three main categories; bacterial and fungal (yeast), or a combination of both [5]. Efficiency of probiotics differs depending upon the probiotic dose rate, diets composition, viable cell number, strains, animal age and stage of growth [6]. According to [7], the main modes of action of yeast probiotics so far identified include: Supplementation of growth factors to rumen micro-organisms, oxygen scavenging that creates more favorable conditions for the anaerobic communities and nutritional competition with autochthonous ruminal microbial species for energy [8]. Furthermore, yeast and bacteria additives can increase the pH and decrease lactate accumulation in the rumen by increasing number of lactate utilizing bacteria, particularly *Selenomonas ruminantium* and *Meganosphera elsdenii* [9] and also by inhibiting the activity lactate-producing bacteria, particularly *Streptococcus bovis* [10]. Yeast supplementation enhances ammonia utilisation by ruminal microorganisms, thus, increased microbial protein synthesis [11]. Certain species of bacteria (*Propionibacteria)* were reported to modify rumen fermentation and increase the molar portion of ruminal propionate [12].

[13] reported that the addition of cell-free supernatant of *L. plantarum* to ruminal samples during short-term batch experiments led to significant increases in volatile fatty acid (VFA) production and significant decreases in methane (CH_4) production, which were accompanied by hydrogen accumulation. These findings suggest that selected species of *Lactobacillus* have the ability to manipulate rumen fermentation. Therefore, the objective of this study was to assess the effect of yeast alone and in combination with *Lactobacillus spp*. (probiotics) on rumen liquor in terms of rumen fermentation characteristics and microbiology of WAD goats.

Materials and Methods

Experimental site

The experiments were conducted at the Departments of Animal Science, Benin, Ibadan and Uyo laboratories in Nigeria

during January, 2014 to December, 2014.

Feed additives used:

The yeast used was bakers' yeast named Angel procured from a supermarket. The mixed probiotic had yeast fortified with *Lactobacillus acidophilus* at a concentration of 1.00 x 10^{12}cfu/g. Samoxine – an antibiotic with oxytetracycline hydrochloride as the active ingredient was used in this study. Probiotic was offered daily (g/day) that is the bakers yeast and mixed yeast plus *Lactobacillus acidophilus*.

Experimental diet:

The concentrate (as seen in Table 1) was formulated and mixed with antibiotic, yeast (at 2.5g and 5g) and mixed probiotic of yeast and LAB (Lactic Acid Bacteria) (at 2.5g and 5g) plus *Panicum maximum* forage as follows:

Diet 1- Unsupplemented concentrate + Forage

Diet 2- Antibiotic supplemented concentrate + Forage

Diet 3- Supplemented concentrate (2.5 g - yeast) + Forage

Diet 4- Suplemented concentrate (5 g - yeast) + Forage

Diet 5- supplemented concentrate (2.5 g – yeast + bacteria) + Forage

Diet 6- supplemented concentrate (5 g – yeast + bacteria) + Forage

Table 1: Gross composition (%) of concentrate feed mixture

Ingredient (%)	%
Dried cassava peel	45
BDG	40.70
PKC	10
Limestone	2.50
Salt	1.50
Vitamin-mineral premix	0.30
Total	100
Calculated CP (%)	10.37
Calculated ME MJ/Kg	2.24

BDG – Brewers Spent Grains;
PKC- Palm Kernel Cake;
CP – Crude Protein;
ME – Metabolizable Energy

Rumen fluid biophysical, biochemical and fermentation characeristics

This experiment was carried out in the Teaching and Research Farm of University of Uyo, Uyo, Department of Animal Science Uniuyo and at PZ Aba. Samples of ruminal fluid was collected from a total of eighteen bucks who were already on the test diets, representing the six diets, just prior to morning feeding with the use of a stomach tube. Immediately after collection, the pH was measured using a pH meter, colour charts was utilized in determining the rumen fluid colour, consistency was observed with feel to fingers and fluid was allowed to sit in a test tube and determine the time (minutes) for complete sedimentation and flotation of solid particles in order to test for sedimentation activity time (SAT). Smaller particles sink, larger particles float on the bubbles of fermentation [14]. To another portion of the rumen fluid, the following parameters were measured: methylene blue reduction time (MBRT) - Add 10 mL of fresh rumen fluid to 0.5 mL of a 0.03% solution of Methylene Blue stain in a test tube and set a timer [15] - and rumen fluid chloride (measured in a supernatant of a centrifuged rumen liquor) and later measured using a spectrophotomer [16].

Eighteen animals used for the growth trial was utilized for the microbial population study [17]. Ruminal fluid sample was taken using stomach tube, prior feeding, to determine population of bacteria, fungi and protozoans using pour plate serial dilution technique and their relevant nutrient agars while identification for bacteria and fungi [18]

and protozoa [19]. The sample was filtered through cheese clothe for Ammonia nitrogen (NH_3-N) concentration to be determined according to the method of [20]. Total Volatile Fatty Acids was determined by steam distillation [21] while the individual fractions of acetate, propionate and butyrate were measured using high-performance liquid chromatography (HPLC).

Statistical design and analysis

The study was conducted in a completely randomised design. All data collected were subjected to analysis of variance using the procedure of [22]. Significant means were separated using the Duncan Multiple Range F-Test. Experimental model of the design is: $Y_{ij} = \mu + \alpha_i + '_{ij}$. Where Yij = Individual observation; μ = general mean of population; αi =treatmen mean; Σij = composite error effect.

Results and Discussion

Table 2 shows the physical, chemical, rumen fermentative characteristics and microbial population of bucks fed probiotic fortified diets. The colour of the rumen liquor was generally brownish green while odour was aromatic and consistency was slightly viscous. The sedimentation activity time was significantly different with D2 recording the highest (6.13 mins) while D4 had the least (4.00 mins). As regards chemical characteristics of rumen fluid, pH ranged from 6.70 to 6.90 and was not significantly different from each other. Methyl blue reduction time was highest for D2 (4.83 mins) and the least was observed in D3 and D5 (4.00 mins). Sedimentation activity test ranged between 4.00 mins in D4 to 6.13 mins in D2. Fluid chloride was highest in D3 (49.13 mEq/L) and least was recorded for D6 (34.00 mEq/L).

Animals on D6 (62.22 mM) recorded the highest total volatile fatty acids while those on D2 (49.67 mM) had the least. Animals on D4, D5 and D6 – 57.50, 59.00 and 62.22 mM - were significantly different from those on other treaments/diets (i.e. D1 – D3) of 49.67 – 54.33 mM. Ammonia nitrogen was highest for animals on D6 (8.15 mg/dl) and the least (4.45 mg/dl) was observed in D3. The mixed probiotic (D5 and D6: 7.85 and 8.15 mg/dl) elicited a higher ammonia nitrogen levels that was comparable ($p > 0.05$) with D2 (7.33 mg/dl) but different ($p < 0.05$) from D1, D4 and D3 (5.91, 4.70 and 4.45 mg/dl). The molar proportion (%) of the volatile fatty acids showed that acetate was highest in D3 (67.88 %) and least in D2 (63.87 %). The propionate was higher in D2 (24.66 %) and the lowest was seen in D5 (19.74 %). The butyrate ranged from 11.28 in D6 to 13.45 % in D5. There was no significant effect of probiotics on lactate concentration in the ruminal fluid. The acetate to propionate ration ranged from 2.55 to 3.47 also showing an influence of probiotics fortification.

Protozoan population in the rumen liquor ranged from 40.00 to 46.00 x 10^3/mL with no significant effect of treatment. However, there were significant differences in the population of bacteria and fungi. Bacteria was highest in animals on D5 (233.33 x 10^6 cfu/mL) and least was seen in those on D2 (129.33 x 10^6 cfu/mL). Animals on probiotic fortified diets (D3, D4, D5 and

D6: 202.67, 205.33, 233.33 and 206.67 x 10^6 cfu/mL) recorded a higher bacteria population than D1 and D2 (175.67 and 129.33 x 10^6 cfu/mL). The fungi population was stimulated by the probiotic fortified diets higher than D1 and D2. Animals on D4 (54.00 x 10^3 cfu/mL) recorded the highest while those on D2 had the least (26.00 x 10^3 cfu/mL).

Table 2: Physical, chemical, rumen fermentative characteristics and microbial population of rumen liquor in probiotics fed WAD bucks

Parameter	D1	D2	D3	D4	D5	D6	SEM
Colour	BG	BG	BG	BG	BG	BG	-
Odour	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	-
Consistency	SV	SV	SV	SV	SV	SV	-
SAT (mins)	5.00^{b}	6.13^{a}	4.67^{c}	4.00^{d}	4.63^{c}	4.17^{d}	0.18
pH	6.70	6.87	6.83	6.90	6.90	6.80	0.13
MBRT (mins)	4.67^{b}	4.83^{a}	4.00^{d}	4.33^{c}	4.00^{d}	4.20^{c}	0.20
RFCl (mEq/L)	42.32^{ab}	42.24^{ab}	49.13^{a}	47.80^{a}	40.27^{ab}	34.00^{b}	3.48
TVFA Mm	54.33^{c}	49.67^{d}	54.33^{c}	57.50^{b}	59.00^{b}	62.22^{a}	0.96
NH_3-N mg/dl	5.91^{b}	7.33^{a}	4.45^{c}	4.70^{c}	7.85^{a}	8.15^{a}	0.28
LACTATE mg/dl	4.05	3.60	4.00	4.05	4.05	4.00	0.18
ACETATE %	66.46^{ab}	63.87^{c}	67.88^{a}	65.98^{ab}	66.81^{ab}	65.22^{b}	0.66
PROPIONATE%	21.33^{cd}	24.66^{a}	20.59^{d}	22.69^{bc}	19.74^{d}	23.50^{ab}	0.55
BUTYRATE %	12.20	11.47	11.53	11.33	13.45	11.28	0.77
Acetate/Propionate	3.12^{b}	2.60^{d}	3.30^{a}	2.91^{c}	3.38^{a}	2.78^{c}	0.05
Protozoan $x10^3$	46.00	40.00	40.67	43.43	44.00	40.33	9.48
Bacteria $x10^6$	175.67^{b}	129.33^{c}	202.67^{ab}	205.33^{ab}	233.33^{a}	206.67^{ab}	9.73
Fungi $x10^3$	28.33^{bc}	26.00^{c}	49.00^{a}	54.00^{a}	50.67^{a}	46.67^{ab}	5.96

a,b,c, = means on the same row bearing different superscripts differ ($p<0.05$) significantly
D1: -ve Control; D2: +ve Control (Antibiotic); D3: Yeast 2.5g/d; D4: Yeast 5.0g/d; D5: LAB+Yeast 2.5g/d and D6: LAB+Yeast 5.0g/d; BG – Brownish green; SV – Slightly Viscous; SAT – Sedimentation Activity Test; MBRT – Methylene Blue Reduction Time; RFCl – Rumen Fluid Chloride; TVFA – Total volatile fatty acids; NH_3-N – Ammonia nitrogen

The results of the effect of probiotic on rumen liqour showed that, the physical characters of the rumen fluid were not influenced between the control group and probiotics fortified groups as color, odour and consistency were brownish green, aromatic and slightly viscous respectively and this result agreed with that of [23] who reported that physical characters of the rumen juice were not changed throughout the experimental period (1^{st} to 8^{th} week) between the control group and probiotics supplemented groups. Also, [24] reported similar results for non-pregnant goats fed probiotic supplemented diets as the color of rumen juice was varied from olive green to brownish green, while the odor was aromatic,. The consistency was slightly viscous to watery. The watery consistency was seen in exogenous enzyme supplemented diet while probiotic supplemented was viscous. The sedimentation activity time (SAT) was prone to the effect of probiotics with higher fortification level showing less time of sedimentation. This result is in agreement with that of [24], where in the 8th week, the probiotic supplemented

diet recorded the least value of 3.25 mins which was similar to enzymatic supplemented diets (4.25 mins) but significantly different from the control (4.75 mins) and this result showed improvement of SAT due to alteration in microbe activity which in turn affected substrate degradation. Normal range for SAT is 4–8 minutes.Very active fluid may exhibit sedimentation of fine particles with subsequent flotation. Inactive fluid shows rapid sedimentation with little to no flotation, due to lack of fermentative gases. Rumen acidosis, prolonged anorexia, indigestible feeds with inactive flora. Stable froth presence indicates frothy bloat or some types of vagal indigestions in the Hoflund disease (stenosis, hypermotility) or in case with treatment with sympathicomimethics (acetylcholine) used for the treatment of ruminal atonia [14].

The pH of the present study was not affected by probiotic fortification as there was no significant difference between the treatments. This assertion was also reported by [24] where there was no alteration in pH level of probiotic, fibrolytic enzyme and control supplemented diets after 8 weeks. They attributed this to the effect of probiotics preventing the accumulation of lactic acid in the rumen and providing a stable environment for rumen fermentation by increasing the pH value [25]. The higher numerical ruminal pH by probiotic fortified diets would be beneficial for making the ruminal environment more favourable for the activity of bacteria (cellulolytic) [26]. The result of this study on pH agrees with earlier findings which stated that probiotics did not affect goats' rumen pH value with any significance [27, 28, 29]. However, it stabilized pH in a range that is compatible with the optimal ruminal ecologic dominance. The methyl blue reduction time (MBRT) is a test for the reducing ability of the anaerobic rumen flora. Normal range for MBRT is 3 – 6 minutes while prolonged discoloration takes longer than 10 – 15 minutes indicating inadequate anaerobic bacterial population, rumen acidosis or indigestible roughage [14]. The result obtained in this study is similar to that reported by [30] who stated that significant prolongation in methylene blue reduction time (reduction time increased) encountered after the second dose of Diarrheastat® and Enrosol-S® indicated inactive ruminal microflora. This assertion is true for animals on antibiotic diet which had significantly reduced ruminal microflora population and in turn, this affected their activity [31]. Rumen fluid chloride main source is the diet and the saliva which passes into the rumen. In case of gastric torsion or in other cases of pylorus obstruction, the hydrogen chloride will pass into the rumen (reflux phenomenon) and can increase chloride up to 30 - 100 mEq/L [14]. In healthy ruminant the ruminal chloride concentration is low (15-20 mEq/L). From this study the probable reason can be attributed to the mode of rumen liquor extraction through use of a tube which might have caused the elevations above normal.

Khadem *et al.* [32] noted that the ammonia nitrogen content was highest in the control over the live yeast supplemented diets with 2.5 g/day being the lowest throughout. Other authors were in agreement with this observation

of reduced ammonia nitrogen after yeast supplementation [33, 34]. In contrast, [35] reported that yeast usage in rations of dairy cows increased their rumen ammonia concentration. The decrease noted in the findings [32] was attributed to the increased incorporation of ammonia into microbial protein production and might be the direct result of the ruminal stimulated microbial activities. This observation is in agreement with the finding of this study. However, increased significant amount of ammonia was observed in D5 and D6 and this indicated that there was greater catabolism of protein and non-protein nitrogen [36]. For animals fed D2, the reason for the increase might have been that some gram-positive bacteria may be resistant to the anitibiotic such as *Clostridium aminophilum* [37]. The concentration for ammonia N fell within the range (5 – 25 mg/dl) reported by [38] as an optimum level of ammonia - nitrogen in rumen fluid for microbial growth except for D3 and D4 which were close to the minimum range level.

The reduction in lactic acid concentration in the rumen liquor due to inclusion of antibiotic in the diet might have been the result of ionophore inhibition of lactate producing bacteria [39]. This is in agreement with the lactate level obtained in this study for animals on D2. The lack of significance in lactate is in agreement with the finding of [40] where yeast at two levels (1 and 2.50g) was compared with antibiotic on high concentrate diet and starch. The lack of response of the probiotics can be attributed to the fact that they act as a lactate utilizing bacteria growth stimulator which are resistant to low pH [41] especially yeast. Therefore their effect would have been significant under lower pH conditions. The high concentrations of total volatile fatty acids (TVFA) for probiotic fortified diets were similar to the findings obtained by several authors [34, 42] for yeast culture supplemented diets. [43] observed a linear increase in total VFA production, as compared to control, with provision of increasing levels of *Lactobacillus casei* and *Lactobacillus lactis* after 6 and 12 h of *in vitro* fermentations; however, no change was observed at 24 and 48h fermentations. [44] reported that in high grain rations, yeast culture (YC) diet supplementation increased ruminal TVFA concentrations, but was not affected by YC when medium or low grain contained rations were used. Hence, increase was adduced to higher rumen microbial activities [45] due to use of YC since they provided soluble growth factors (organic acids, B vitamins and amino acids) for ruminal microbes which may stimulate their growth and activities. The reduced TVFA for D2 can be associated with the decreased microbial activity and growth.

In vitro studies, yeast probiotics has beneficial effects on growth and H_2-utilisation of acetogenic bacteria as observed by [10] and since the acetogenic bacteria, which produce acetate from CO_2 and H_2, the acetic centesimal proportion and/or total VFA produced in the rumen should appear to increase. However, in an *in vivo* experiment that was carried out in lambs [46], even though total VFA was significantly higher in the *S. cerevisiae* group during the 20–50 d period, no

significant effect was observed on the centesimal composition of the major VFA mixture (acetate, propionate, and butyrate) except that of acetate tended to increase. This was observed in this study as acetic acid tended to increase over the other acids. [47] reported that the supplementation of *L. acidophilus* was shown to increase in ruminal propionate concentrations. Moreso, the report [48] of increasing levels of probiotics (*L. acidophilus* 2 x 10 12; *S. cerevisiae* 5 x 10 11 cfu/g at 0, 2.5, 5.0 and 7.5 g/h/day) eliciting an increased ($p < 0.05$) acetic centesimal proportion while butyrate was unaffected by the probiotic supplementation was similar to this study. However, their propionate concentration decreased with increase inclusion of probiotics contrary to the findings of this study.

Presented in Table 3 are the identified protozoans, bacteria and fungi. The identified protozoans were *Isotricha intestinalis, Dasytricha ruminantium, Entodinium vorax and Diplodinium medium.* The bacteria found within the different animal treatments were mostly *Bacillus spps, Streptococcus faecalis, Corynebacterium spp, Alcaligenes faecalis*, *and Lactobacillus spps.* Others identified were *Micrococcus spp., Salmonella paratyphi Shigella spp., Aerococcus viridans, Pediococcus cerevisiae, Enterobacter spp., and Erwinia herbicola.* The fungi found were mostly *Aspergillus species* together with *Saccharomyces cerevisiae* (in animals on D3, D4, D5 and D6), *Microsporum spp., Fusarium oxysporum, Penicillium expansium and Fusarium spp.*

Several authors [49, 50] have reported that the supplementation of probiotics had no significant effect on protozoa population but on the contrary, [51] found an increase of protozoal count by the occasion of addition of *S. cerevisiae.* [47] stated that supplementation of *L. acidophilus* has been shown to increase ruminal protozoal numbers. In the same vein, [27] reported the significant increment of protozoal and bacterial counts for the reason of supplementation of blend of *S. cerevisiae* and *L. acidophilus* probiotics. The results of this study were similar to the findings from [47] and [27] except that protozoan numbers decreased as against control and fungal population increased for probiotic fortified diets when compared with control. Thus, it can be said that probiotic has manipulative effect on rumen microorganisms.

Eleven organisms were identified as gram positive bacteria throughout the rumen liquor of the animals under study. These are: *Aerococcus viridans, Micrococcus spp., Staphylococcus spp., Streptococcus faecalis, Pediococcus cerevisiae, Clostridium bifermentans, Corynbacterium xerosis, Listeria monoctogenes, Lactobacillus spp.*, and *Bacillus spp*. (also *B. polymyxa, B. subtilis, B. pumilis* and *B. cereus*). The gram negative bacteria identified were: *Proteus mirabilis, Bacteroides spp., salmonella paratyphi, Escherichia coli, Yersinia pestis, Shigella sonnei, Klebsiella ozaenae, Enterobacter spp., Erwinia herbicola*, and *Alcaligenes faecalis*. The control diet (D1) alone had *E. coli* in its rumen liquor. The *Bacteroides* are efficient in cellulolytic, hemicellulose and starch (pectinolytic and amylolytic) degradations while

Streptococcus bovis are good in starch (pectinolytic and amylolytic). Protein degraders are *Clostridium bifermentans, Streptococcus bovis, Bacteroides ruminicola* and *Bacteroides amylophilus* while sugar utilizers are *Lactobacillus spp*. *Micrococcus spp* has been noted to be a lipid utilizing bacteria also *Anaerovibrio lipolytica.* Ammonia producers are *Bacteroides spp, Selenomonas ruminantium*. The protozoans identified were similar to that found in ruminants [52].

Table 3 : Identified protozoan, bacteria and fungi in rumen liquor of WAD bucks fed probiotics

Protozoa	**Identified species**	
D1 – D6	*Isotricha intestinalis, Dasytricha ruminantium, Entodinium vorax and Diplodinium medium*	
	Bacteria	**Fungi**
D1	*Micrococcus spp., Bacillus subtilis, Lactobacillus spp., Strep tococcus faecalis, Salmonella paratyphi, Escherichia coli, Shigella dysenteriae, Listeria grayii, Corynebacterium uberies,Bacteroides,Clostridium bifermentans, Staphylococcus spp*	*Aspergillus niger, Aspergillus fumigatus, Microsporum spp., Aspergillus glauc us, Fusarium oxysporum, Penicillium frequentans, Rhizopos stolonifer*
D2	*Bacillus spp., Proteus mirabilis, Listeria monocytogenes, Aerococcus viridans, Corynebacterium monocytogenes*	*Aspergillus terreus, Aspergillus niger, Monilla spp., Humicola spp.*
D3	*Pediococcus cerevisiae, Chromobacterium marismortui, Streptococcus faecalis, Bacillus cereus, Corynebacterium monocytogenes, Bacteroides*	*Aspergillus terreus, Penicillium expansium, Verticillium alboatrum, Fusarium oxysporum, Saccharomyces cerevisiae*
D4	*Alcaligenes faecalis, Corynebacterium xerosis, Bacillus pumilus, Bacillus cereus, Erwinia herbicola, Listeria grayii, Corynebacterium uberies, Streptococcus faecalis, Bacteroides, Clostridium bifermentans*	*Saccharomyces cerevisiae, Penicillium expansium, Aspergillus fumigatus, Aspergillus flavus, Penicillium frequentans, Phoma spp. , Rhizopos stolonifer*
D5	*Bacillus polymyxa, Streptococcus faecalis, Alcaligenes faecalis, Enterobacter spp., Bacillus cereus, Lactobacillus spp., Bacteroides, Corynebacterium uberies*	*Rhodotonila spp., Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Botrytis spp., Saccharomyces cerevisiae*
D6	*Klebsiella ozaenae, Yersinia pestis, Shigella sonnei, Salmonella spp., Bacillus cereus, Bacteroides, Corynebacterium spp., Strept ococcus faecalis, Lactobacillus spp,Clostridium bifermentans*	*Aspergillus niger, Penicillium expansium, Verticillium spp., Fusarium spp., Saccharomyces cerevisiae*

D1: -ve Control; D2: +ve Control (Antibiotic); D3: Yeast 2.5g/d; D4: Yeast 5.0g/d; D5: LAB+Yeast 2.5g/d and D6: LAB+Yeast 5.0g/d

Conclusion and Application

1. In conclusion, probiotics certainly improved the rumen functioning of ruminants which can invariably lead to better growth and health.
2. The manipulative effect of probiotics elicited an improvement in microbiological population and prevalence.

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