BAP -24

Influence of Probiotics on Rumen Liquor Characteristics and Microbiology

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Abstract
Probiotics an alternative to antibiotics 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. 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, Baker's yeast, *Lactobacillus acidophilus*, rumen liquor, microbiology

Introduction

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 (Seo *et al*., 2010). 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 (Barton, 2000). 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.

The term probiotic has been defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). Efficiency of probiotics differs depending upon the probiotic dose rate, diets composition, viable cell number, strains, animal age and stage of growth (Chaucheyras-Durand *et al*., 2008). Furthermore, yeast and bacteria additives can increase the pH and decrease lactate accumulation in the rumen (Callaway and Martin, 1997). Yeast supplementation enhances ammonia utilisation by ruminal microorganisms, thus, increased microbial protein synthesis (Chaucheyras-Durand *et al*., 2008). Certain species of bacteria (*Propionibacteria*) were reported to modify rumen fermentation and increase the molar portion of ruminal propionate (Stein *et al*., 2006). These findings suggest the manipulative effect on 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

The experiments were conducted at the Departments of Animal Science Benin, Ibadan and Uyo laboratories in Nigeria. The yeast used was bakers' yeast named Angel. The mixed probiotic had yeast fortified with *Lactobacillus acidophilus* at a concentration of 1.00×10^{12}cfu/g. Samoxine – an antibiotic with oxytetracycline hydrochloride as the active ingredient was used in this study. Probiotic was offered daily (g/day). 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- Supplemented 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

Samples of ruminal fluid were collected from a total of eighteen bucks 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 sedimentation activity time (SAT) (Rosenberger, 1977). To another portion of the rumen fluid, the following parameters were measured: methylene blue reduction time (MBRT) as described by Coles (1986) - and rumen fluid chloride measured using a spectrophotomer (Dirksen and Smith, 1987). The population of bacteria, fungi and protozoans was determined using pour plate serial dilution technique and their relevant nutrient agars (Galyean, 1989). The sample was filtered through cheese clothe for Ammonia nitrogen (NH_3-N) concentration to be determined according to the method of (Conway, 1957). Total Volatile Fatty Acids was determined by steam distillation (A.O.A.C., 1990) while the individual fractions of acetate, propionate and butyrate were measured using high-performance liquid chromatography (HPLC).The study was conducted in a completely randomised design.

All data collected were subjected to analysis of variance using the procedure of (SAS, 1999). Significant means were separated using the Duncan Multiple Range F-Test.

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. Animals on D6 (62.22 mM) recorded the highest total volatile fatty acids while those on D2 (49.67 mM) had the least. Ammonia nitrogen was highest for animals on D6 (8.15 mg/dl) and the least (4.45 mg/dl) was observed in D3. There was no significant effect of probiotics on lactate concentration in the ruminal fluid. 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 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) for fungi population.

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 colour, odour and consistency were brownish green, aromatic and slightly viscous respectively and this result agreed with that of Salem *et al*. (2002). The SAT result is in agreement with that of (Ismael *et al*., 2014). Normal range for SAT is 4–8 minutes. Very active fluid may exhibit sedimentation of fine particles with subsequent flotation.

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 pH of the present study was not affected by probiotic fortification and this assertion was also reported by (Ismael *et al*., 2014) where there was no alteration in pH level of probiotic, fibrolytic enzyme and control supplemented diets. 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 (Rosenberger, 1977). This assertion is true for animals on antibiotic diet which had significantly reduced ruminal microflora population and in turn, this affected their activity (Das, 1992). Khadem *et al.* (2007) noted that the ammonia nitrogen content was highest in the control over the live yeast supplemented diets and this observation is in agreement with the finding of this study. 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 (Russell and Strobel, 1989). This is in agreement with the lactate level obtained in this study for animals on D2. The high concentrations of total volatile fatty acids (TVFA) for probiotic fortified diets were similar to the findings obtained by several authors (El-Ghani, 2004; Arcos-Garcia *et al*., 2000) for yeast culture supplemented diets. Han *et al*. (2008) reported significant increment of protozoal and bacterial counts for the reason of supplementation of blend of *S. cerevisiae* and *L. acidophilus* probiotics. Thus, it can be said that probiotic has manipulative effect on rumen microorganisms.

Conclusion

In conclusion, probiotics certainly improved the rumen functioning of ruminants which can invariably lead to better growth and health. The manipulative effect of probiotics elicited an improvement in microbiological population and prevalence especially for animals fed diets supplemented with yeast and *Lactobacillus acidophilus* at 2.50 and 5 g/d.

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