
EFFECT OF ENZYME SUPPLEMENTATION ON RUMEN ENVIRONMENT OF SHEEP FED A CONCENTRATE DIET

F. I. Abbator, U. M. Kolo, A. M. Said., Z. M. Chana, I. g. Asheikh , S. Daniel. And A. U. Sanda
Department of Animal Science, University of Maiduguri, Maiduguri, Nigeria

ABSTRACT

The study was conducted to determine the effect of exogenous enzyme supplementation on rumen count, isolation and identification of bacteria in sheep fed a concentrate diet. Twenty (20) sheep weighing on average 22.65kg were used for the study. The animals were weighed and divided into four (4) groups. Each group of five (5) animals was randomly assigned to one of the treatments in a Complete Randomized Design (CRD). Rumen liquor was collected from three (3) animals in each treatment for the rumen bacterial count. The enzyme was included at the levels of 0, 200, 400 and 600g in T1 (control), T2, T3 and T4 respectively. The result of the study showed that the bacterial species isolated and identified from the rumen liquor were four of fiber digesting bacteria (*Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus albus* and *Corynebacterium* spp.) and three amylolytic bacteria (*Bacillus subtilis*, *Klebsiella* spp. and *Proteus mirabilis*). The rumen bacterial count differed significantly ($P < 0.05$) among the treatments, while supplementation with exogenous enzyme had no effect ($p > 0.05$) on the rumen pH. The result showed that sheep on concentrate diet only (T1) had significantly ($p \leq 0.05$) lower bacterial count, while bacterial count of sheep on T2, T3 and T4 increased ($p \leq 0.05$) as the level of supplementation with the enzyme increases. In conclusion, the result of the study showed that supplementation with enzyme increased rumen bacterial count and had no effect on the rumen pH

Keywords: Corn cob, sheep, rumen environment, enzyme

Introduction

Livestock production in the tropics and subtropics is mostly influenced by seasonal scarcity and low quality of feed resources. In Africa, small ruminants make a substantial contribution to the wellbeing of the people in the region through the supply of meat, milk, fibre, drawn power, manure and cash (Deleeuw and Ray, 1995). Despite their low productivity due to genetic or

environmental constraint or both, small ruminants play an important role in the agricultural economy of sub-Saharan Africa as indicated by Winrock International (1983).

Several tons of crop residues capable of feeding millions of livestock such as cattle, sheep and goats are produced annually in Nigeria. Crop residues have been estimated to account for about 25% of the total feed energy suitable for ruminants livestock in both developed and developing countries (Kossila, 1985). It has been important in ruminant feeding, however, when used alone they have a very low feeding value with poor metabolisable energy, negligible available protein and seriously deficient in minerals and vitamins (Staniforth, 1979). However, improved utilization of crop residues can be achieved either through appropriate supplementation (legumes, urea, etc.) or chemical treatment (urea/ammonia) both of which facilitate the microbial breakdown of the cell wall of the crop residues.

The exogenous enzymes have shown promise at hydrolyzing plant cell walls (Bhat and Hazlewood, 2001) and revealed new opportunities to improve feed utilization in animals' nutrition (Sheppy, 2001). Supplementing diets with enzymes has been shown to improve feed efficiency and daily gain Bala *et al.* (2009) observed an increase in milk yield of lactating goats supplemented with enzymes. Enzyme supplementation increases the average daily gain by 51 and 69% in sheep and goats respectively (Salem *et al.*, 2011). Enzyme addition tends to increase the total viable bacterial count (Colombatto *et al.*, 2003). In a study reported by Nsereko *et al.* (2002), inclusion of an exogenous fibrolytic enzyme in dairy cows diets increased the number of rumen bacteria. Similarly, Mao *et al.* (2013) reported increased total number of bacteria in the rumen. Therefore, the supplementation of animal feeds with enzymes to increase the efficiency of digestion can be seen as an extension of the animal's digestive process (Sheppy, 2001). The objective of the study is therefore to determine the effect of supplementation with exogenous enzyme on rumen count, isolate and identify bacterial species in sheep.

Materials and Methods

Location of the Study Area

The study was conducted at the Department of Animal Science Teaching and Research Farm, University of Maiduguri. Maiduguri is located between latitude 11⁰⁵¹ and 12⁰ North and Longitude 13⁰⁵¹ and 14⁰ East and at altitude of 354m (1161ft) above sea level (DNMA, 2013). The area falls within the semi-arid zone of West Africa characterized by short duration of rainfall (3-4 months) which varies from minimum of 478mm to 500mm to maximum of 600mm-621mm (Afolayan *et al.*, 2013). The mean temperature is 34⁰C, the maximum being 40-60⁰C and the lowest 25⁰C, which is in April and December, respectively.

Animals and Experimental design

Twenty (20) sheep of non-descript breed weighing on average 22.65 kg were used. All the animals were obtained from the flock of sheep kept at the Department of Animal Science Livestock Teaching and Research Farm, University of Maiduguri. They were weighed and identified using plastic ear tags. Feeding was done at 4% body weight once daily at 8:00 am with the left over being weighed before the next feeding. The animals were divided into 4-groups and each group of 5-animals was randomly assigned to one of the treatments in a completely randomized design (CRD). The study lasted for 11 weeks.

Treatments (Experimental diets)

The feed ingredients used for the formulation Of the experimental diets were maize cob, wheat offal, cotton seed cake, poultry litter and exogenous enzyme. The diet formulated consisted of maize cob (40%), wheat offal (30%), cotton seed cake (15%), poultry litter (15%). The enzyme was included in the diet at levels of 0, 200, 400 and 600 g in T1 (control), T2, T3 and T4 respectively.

Table 1: The composition of the Experimental Diets (%)

Ingredients	Treatments and Level of Enzyme Supplementation (g)			
	T1(0)	T2(200)	T3(400)	T4(600)
Corn cob	40	40	40	40
Wheat offal	30	30	30	30
Cotton seed cake	15	15	15	15
Poultry litter	15	15	15	15
Total	100	100	100	100

Rumen Liquor Collection

The left para lumber posa was shaved and aseptically prepared with chlorhexidine-gluconate. The animal was physically restrained in a standing position. Anaesthesia was achieved using 2% Lidocaine infiltrated in a ring block pattern. A long aspirating needle was passed through the dorsal part of the rumen until a foul smell was perceived. An ingress tube to which a 10 ml springe was attached was connected to the aspirating long needle. Using the plunger, 10 ml of rumen liquor was then collected and poured into a sterile container with the pH immediately recorded. The samples were then taken to the Veterinary Medicine Laboratory, University of Maiduguri for isolation and identification of rumen bacteria.

Statistical Analysis

The data generated were subjected to analysis of variance (ANOVA) using the complete randomized design (CRD) and the Duncan multiple range test was used for the mean separation.

RESULTS AND DISCUSSIONS

Table 2. Proximate Composition (%) of Feed Ingredients

Ingredients	DM	CP	EE	CF	Ash	NFE
Cotton seed cake	96.30	15.10	8.00	23.00	5.00	48.90
Wheat offal	94.30	8.31	3.00	19.00	5.00	64.69
Poultry litter	95.20	10.11	3.00	25.00	4.00	57.89
Maize cob	96.10	5.07	3.00	21.00	4.00	66.93

DM = Dry Matter, CP = Crude Protein, EE= Ether Extract, CF = Crude Fibre, NFE = Nitrogen Free Extract

The results of proximate composition of the feed ingredients were shown in Table 1. The results showed that cotton seed cake had the highest dry matter content (96.3%), which is higher than 92.6% and 91.6% reported respectively by Ehoche, (1982) and Solaiman (2007). Wheat offal had the lowest dry matter content (94.30%) which is higher than 86.10% and 90.0% reported respectively by Bello (1984) and Amaetule *et al.* (2016). Cotton seed cake had the highest crude protein content (15.10%) which is lower than the value 48.5% reported by Ehoche (1982) and slightly higher than 14.0% reported by Solaiman (2007). The differences could be due to method of processing. Maize cob had the lowest crude protein value (5.07%) and however, the value recorded is higher than 3.0% and 4.4% reported by Ososanya *et al.* (2013) and Heuze *et al.* (2015) respectively. This could be due to varietal difference. The ether extract content recorded for cotton seed cake in this study was (8.0%) which is higher than 3.06% and 4.5% reported respectively by Ehoche (1982) and Solaiman (2007). The ether extract content of poultry litter, maize cob and wheat offal were similar (3.0%). However the value is higher than 1.30% reported by Bello (1984) for wheat offal but slightly lower than 4.35% reported by Amaetule *et al.* (2016). Also it is higher than 1.5% reported by Bolan *et al.* (2010) for poultry litter and higher than 1.8% and 1.1% reported respectively by Ososanya *et al.* (2013) and Heuze *et al.* (2015) for maize cob. Poultry litter had the highest crude fibre content (25%) which is lower than 15% reported by Bolan *et al.* (2010). The lowest value is recorded in wheat offal (19%) which is higher than 11.5% and 7.0% reported respectively by Bello (1984) and Amaetule *et al.* (2016).

Cotton seed cake and wheat offal had the highest ash content (5.0%) which is comparable with the values 5.8% and 5.53% reported respectively by Amaetule *et al.* (2016) and Solaiman (2007) for wheat offal and cotton seed cake. However the value is slightly lower than 6.75% reported by Ehoche (1982). This could be attributed to mineral availability in the soil where these plants were grown.

Table 3. Proximate Composition (%) of Experimental Diet

Treatments	DM	CP	EE	CF	Ash	NFE
T1	95.20	18.25	16.00	5.00	4.00	56.75
T2	95.80	21.35	18.00	5.00	4.00	51.65
T3	96.30	17.65	18.00	6.00	4.00	54.35
T4	96.40	16.34	21.00	5.00	5.00	52.60

DM = Dry Matter, CP = Crude Protein, EE= Ether Extract, CF = Crude Fibre, NFE = Nitrogen Free Extract

Proximate Composition of Experimental Diets

The proximate composition of the experimental diets is presented in Table 2. The crude protein values were 18.25, 21.35, 17.65 and 16.34% for T1, T2, T3 and T4 respectively. T2 had the highest value (21.35%) and the lowest value is recorded in T4 (16.34%). The crude fibre values were 16.0, 18.0, 18.0 and 21.0% for T1, T2, T3 and T4 respectively. T4 recorded the highest value (21.0%) and T1 had the lowest value (16.0%) while T2 and T3 values were similar in crude fibre value (18.0%). The ether extract values ranged from 5.0%-6.0%, T3 recorded the highest value (6.0%) while the values for the other diets were similar. The ash ranged from 4.0% - 5.0%. T1, T2 and T3 were similar in ash value.

TABLE 4: Bacterial species isolated from the rumen liquor of sheep

Bacteria	Gram Stain	T1	T2	T3	T4
<i>Klesiellaspp</i>	-	P	⊘	P	⊘
<i>Proteus mirabilis</i>	-	P	⊘	⊘	⊘
<i>Staphylococcus albus</i>	+	⊘	P	P	P
<i>Bacillus subtilis</i>	+	⊘	P	P	P
<i>Escherichia coli</i>	-	P	P	P	P
<i>Streptococcus foecolis</i>	+	⊘	P	⊘	P
<i>Corynebacterium spp</i>	+	P	P	⊘	P

+ = gram positive

- = gram negative

P=present

⊘ =not detected

Bacterial species isolated

Bacterial species isolated from the rumen liquor of sheep were shown in table 3. The results obtained showed that *Escherichia coli* occurred in all the treatments. This might be due to the high proportion roughage in the diet. *Escherichia coli* increase with increase in content of roughages in ruminant diets (Rasmussen *et al.*, 1993). *Corynebacterium spp.* also occurred in all the treatments. *Corynebacterium species* ferment starchy feeds in the rumen to propionic acid (Gutierrez, 1953). The high population of *corynebacterium* might be attributed to the concentrate diet used in the feeding trial. Similarly, *Streptococcus species* ferment starchy as reported by McPherson *et al.* (1953). *Proteus mirabilis* had the least occurrence in T1. The kind of microbes synthesized in the rumen depends on the type of feed consumed (Kudva, 1997). All species of bacteria from the rumen liquor were the normal flora of rumen microbes of ruminants under tropical conditions (Hespell, 1981). However it is believed that ruminal fermentation primarily by changing ruminal microbial populations (Ives *et al.*, 2002). Nagaraja and Titgemeyer (2007) assumed that ruminal bacteria react to amplified availability of fermentable substrates by increasing growth rates and fermentative activities, which leads to the improved production of ruminal fermentation.

Table 5. Rumen Bacterial Count in sheep

Parameters	T1	T2	T3	T4	SEM
Bacterial count	8,000 ^a	200,00 ^{ab}	24,000 ^{ab}	27,000 ^b	0.026*
pH	6.57 ^a	6.19 ^a	6.10 ^a	6.31 ^a	0.870 ^{NS}

a,b means in the same row varied significantly at ($p \leq 0.05$) from each other. Rumen Bacterial Count

The results of the rumen bacterial count and pH were shown in Table 3. The rumen bacterial count differed significantly ($P < 0.05$) among the treatments while, supplementation with exogenous enzyme had no effect ($p > 0.05$) on the rumen pH. The results showed that T1 (control) had the least bacterial count, the low bacterial count in T1 justified the need for supplementation with enzyme. T4 had the highest bacterial count among the treatments. This could be attributed to the highest level of enzyme supplementation. The result is in conformity with the work of Nsereko *et al.* (2000) and Mao *et al.* (2013). T2 and T3 were similar ($p > 0.05$) in bacterial count however; the bacterial count in T1 was lower than T2 and T3. The bacterial count increased as the level of inclusion of the enzyme increased. The result of this study agrees with Colombatto *et al.* (2003) who reported increase in bacterial count as the level of supplementation with enzyme increases. The highest pH is recorded in T1 and the lowest The optimal pH for normal bacterial growth in the rumen ranged from 6.0-7.0 (Flint *et al.*, 2008). The diets with increasing levels enzyme including were designed to increase the amount of readily fermentable feedstuffs and to reduce pH in the rumen. However, the ruminal pH of was relatively high throughout the trial. The diet did not provide any clinical signs of acute or subacute acidosis (Garrett, 1996) in any animal during this study.

Conclusion

The result of the study showed that the bacteria species isolated and identified from the rumen liquor of the experimental animals included four fiber digesting bacteria (*Escherichia coli*, *Streptococcus foecolus*, *Staphylococcus albus* and *Corynebacterium spp.*) and three amylolytic bacteria (*Bacillus subtilis*, *Klebsiella spp.* and *Proteus mirabilis*). More research should be conducted using enzymes in ruminant diets to determine their modes of action in rumen in order to increase feed efficiency and enhance performance. The result of the study showed that inclusion of exogenous enzyme in the diet at varying levels increased the rumen bacterial count

(number) and had no effect on the rumen pH. More research should be conducted to determine the effect of exogenous fibrolytic enzymes on rumen microbes in cattle and goats.

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