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PERFORMANCE CHARACTERISTICS AND RUMEN MICROBIAL POPULATION OF WEST AFRICAN DWARF SHEEP FED ENZYME SUPPLEMENTED DIETS

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ABSTRACT

A 56-day trial was conducted to determine the effect of exogenous fibrolytic enzyme on growth performance and rumen microbial population of West African dwarf sheep. Sixteen growing ewe were randomly subjected to four dietary treatments with four animals per treatment in a completely randomized design. A total mixed ration was formulated with enzyme included at four levels: 0, 0.2, 0.4 and 0.6 g/kg feed. Performance characteristics and rumen microbial population of the animals were determined. Enzyme additive significantly influenced ($P<0.05$) weight gain, feed intake and feed conversion ratio. The highest values for total weight gain, and total feed intake were obtained at 0.6g/kg enzyme inclusion level. The feed conversion ratio (12.63) was however lowest ($P<0.05$) at 0.4g/kg enzyme inclusion. The total bacteria and total fungi counts were also significantly ($P<0.05$) affected by the dietary treatments. The highest bacteria (4.73×10^6 cfu/ml) and fungi counts (3.27×10^6 cfu/ml) were obtained at 0.6g/kg enzyme inclusion level but these values were not significantly different from what was obtained at that of 0.4g/kg enzyme inclusion. Protozoa count was not significantly ($P>0.05$) affected by the dietary treatments. A total of eight bacteria were isolated in the rumen of the animals. It is therefore concluded that the enzyme additive improved the performance and microbial count of sheep when added at 0.4g/kg feed.

Keywords: Performance, West African Dwarf Sheep, Total Mixed Ration, Enzyme Additive

INTRODUCTION

The ability of ruminants to convert plant materials unsuitable for human consumption into high protein meat and milk is of great importance to livestock producers. However, the efficiency by which this occurs is largely determined by the digestibility of plant cell wall; cellulose, hemicellulose and lignin. Low forage digestibility continues to limit the intake of available energy and protein by ruminants and contribute to excessive nutrient excretion by livestock (Beauchemin *et al*., 2003). Ruminants depend on the microbial flora in the rumen to digest cellulose. Digestion of feed in the rumen is primarily carried out by the rumen micro flora which contains dense populations of several species of bacteria, protozoa, yeast and fungi. It is estimated that 1mm of rumen contain 10-50 billion bacteria, 1 million protozoa, several yeasts and fungi (Hackmann and Spain, 2010). Supplementing ruminant diets with exogenous enzymes has shown high potential to improve cell wall digestibility and thus, the efficiency of feed utilization and increased digestible en-

ergy intake (Beauchemin *et al*., 2003). Therefore, the selection of enzymes for use as ruminant feed additives should be first tested in a ruminal environment (Colombatto and Beauchemin, 2003). It is also important to test enzymes on individual feeds, as their activity may be specific to the type of feed **.**

Enzymes are added to ruminant diets to supplement fibrolytic activity in the rumen, stimulating dry matter degradation and eventually microbial numbers. Several research had been done on efficacy of exogenous fibrolytic enzyme in fibre degradation (Feng *et al*., 1996, Yang *et al*., 1999), milk production (Yang *et al*., 2000) and growth (McAllister *et al*., 1999). According to Morgavi *et al*. (2000) exogenous fibrolytic enzymes work in synergy with endogenous rumen microbiological enzymes to enhance digestibility and nutritive value of a high fibrous diet. There is need to assess the effect of exogenous fibrolytic enzyme on rumen microbes which are involved in feed degradation in the rumen and also to complement the information available on feed intake and weight gain. Hence, the objective of this study was to determine the feed intake, weight gain, feed conversion ratio and rumen microbial population in West African dwarf sheep fed supplemented diets.

MATERIALS AND METHODS

Location of the Experiment

The study was carried out at the small ruminant unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun state while the rumen microbial population was determined at the laboratory of the Department of Veterinary Microbiology of the same University. Ogun state is in the rainforest zone of Southwestern Nigeria. The area has an annual mean temperature of 34.7 ^{0}C, a relative humidity of 82% and an annual mean rainfall of 1037 mm. It is in the region 70 m above sea level and lies on latitude 7^0 5' - 7^0 8' N and longitude 3^0 11.2' E.

Experimental Diets / Experimental Animals Management

The test ingredient was an exogenous fibrolytic enzyme, ROXAZYME G2$^{(R)}$ which contains Cellulase, betaglucanase and Xylanase. The enzyme was obtained commercially. A total of four experimental diets were formulated with the enzyme included at 0, 0.2, 0.4 and 0.6 g/kg of the Total Mixed Ration (TMR) (Table 1). Total Mixed Ration is a homogenous mixture of ration ingredients that typically combine roughages (forages) and concentrate such as grains to optimize animal performance. Maize Stover which contributed 40% to the TMR was collected at a maize plantation (the maize Stover was left on the field as standing hay) after harvesting within the premises of the university, dried in the sun for two days and crushed with the aid of hammer mill before incorporation into the diet.

A total of sixteen female growing WAD sheep of 6-7 months of age weighing between 10 and 14 kg were randomly assigned to four experimental diets having four replicates per diet in a completely randomized design. The pens were thoroughly washed and disinfected. The sheep were treated against endo and ecto-parasites before the commencement of the experiment. The animals were allowed two weeks of adaptation before data collection. Initial weight of the animals was taken at the beginning of the experiment and thereafter at weekly basis to determine weight changes. The diets were offered at 9.00 am daily at 5% of the ani-

mals' body weight and clean water was offered daily *ad libitum.* Leftover feed was weighed and discarded the following morning. Voluntary feed intake was determined as the difference between feed offered and feed refused. The experiment lasted for eight weeks.

Table 1: **Composition (%) of the experimental diet (Total Mixed Ration)**

Ingredients	T1	T2	T3	T4
Maize Stover	40.00	40.00	40.00	40.00
Maize	10.00	10.00	10.00	10.00
Wheat offal	25.00	25.00	25.00	25.00
Soyabean meal	10.00	10.00	10.00	10.00
Palm kernel cake	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00
Salt	2.00	2.00	2.00	2.00
ROXAZYME G2	_	+	++	+++
Total	100.00	100.00	100.00	100.00

- = 0 g/kg (T1), + = 0.2 g/kg (T2), ++ = 0.4 g/kg (T3), +++ = 0.6 g/kg (T4) enzyme inclusion levels

Collection and analysis of rumen liquor

Rumen fluid was collected from sheep in each treatment before the experiment and at the end of the experiment before morning feeding for the determination of microbial population and identification. About 10 mL of rumen fluid was taken via the oesophagus with the aid of suction tube. It was made free of coarse particles by filtration with cheese cloth, pour in a well labeled sample bottle and taken immediately to the laboratory for the determination of pH and microbial population. Viable bacteria count was done as established by Brown *et al.* (1989) whereas identification of the suspected bacteria was carried out according to Cowan and Steel (1993). Total fungi count determination was carried out as reported by Joblin (1981) and total protozoa count according to Ogimoto and Imai (1981).

Chemical / statistical analysis

Feed sample was analysed for proximate composition (crude protein, crude fibre, ether extract and ash content) using AOAC (2005). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were also determined (Van Soest *et al*., 1991). Cellulose and hemicellulose were calculated as differences between ADF and ADL, and NDF and ADF respectively. Data collected were subjected to one-way analysis of variance while significant differences among means were separated using Duncan's Multiple Range F-test (Duncan, 1955).

RESULTS AND DISCUSSION

The chemical composition of the total mixed ration fed to WAD sheep (Table 2) in this study revealed that the diet contained the required nutrients for sheep. The DM value (93.88%) observed was considered adequate

as it is a reflection of the nutrient content of feed aside water. The CP of 14% is in line with the report of Aduku (1993) that determined the CP requirement of sheep to be 9-14%. Kearl (1982) reported a range of 11-13% for maintenance and growth of sheep and goats. The crude fibre (CF) value (23.02%) of the diet was high. This is not unconnected with the ingredients composition of the diet, maize stover, a crop residues with crude fibre content of 33.2% (Aduku, 1993) made up of 40% of the diet. The ether extract and ash content of the diet were high which showed that the diet was rich in fat and minerals. The NDF (90.12%), ADF (75.16%) and ADL (46.22%) values were high and invariably the cellulose and hemicellulose values. This meant that the diet was high in cell wall content and if the cell wall content (cellulose, hemicellulose and lignin) were well digested by the animals, they will add to the energy content of the diet. According to Belewu *et al.* (2008), moderately high NDF and ADF make more energy available to ruminants

Table 2: Chemical composition (%) of the total mixed ration

Parameters	% Composition
Dry matter	93.88
Crude Protein	14.00
Crude fibre	23.02
Ether extract	8.45
Ash	8.20
Neutral Detergent Fibre	90.12
Acid Detergent Fibre	75.16
Acid Detergent Lignin	46.22
Cellulose	28.94
Hemicellulose	14.96
Gross energy (MJ/kg DM)	15.67

Means of three determinations

Inclusion of exogenous fibrolytic enzyme at different levels influenced the performance indices measured (Table 3). The final weight, total weight gain and daily weight gain of sheep fed diet T4 across the treatments were not significantly different from those fed with T3 while the least daily weight gain and total weight gain was observed in animals fed control diet (T1). The improvement observed in weight gain was in agreement with the report of Zahiroddini *et al*. (2004) that fibrolytic enzymes enhanced weight gain in awassi lambs. It was also in line with the report of Yusuf *et al*. (2013) and El-kady *et al.* (2006), who reported that the use of enzyme treatment in concentrate feed when feeding poor quality forage had a positive effect on weight gain of yearling cross bred calves and growing Buffalo calves respectively. Improvement was observed on daily feed intake, total feed intake as well as FCR in the sheep fed TMR with enzyme additives when compared with those on control diet. Significantly higher feed intake was obtained in sheep fed T4. This was in agreement with the report of Titi

and Tabba (2004) that fibrolytic enzyme increase feed intake of animals fed enzyme-based diets when compared with those on control diet. Sheep fed TMR with enzyme additives recorded lower FCR compared to those on control. This indicated that fibrolytic enzymes improved the sheep conversion ratio and hence, could enhance the growth of the sheep. This observation was in tandem with the report of Titi (2004) who found that exogenous fibrolytic enzyme resulted in improved feed conversion ratio of fattened Awassi sheep with no effect on feed intake.

Table 3: Performance characteristics of West African Dwarf sheep fed total mixed ration with enzyme additive

Parameters	T1	T2	T3	T4	SEM
Average initial weight (kg)	12.50	12.70	13.00	12.60	0.09
Average final weight (kg)	13.79^{c}	14.52^{b}	15.05^{a}	14.86^{a}	0.13
Total weight gain (kg)	1.29^{b}	1.82^{ab}	2.05^{ab}	2.26^{a}	0.14
Daily weight gain (g/d)	23.03^{b}	32.50^{ab}	36.60^{ab}	40.36^{a}	2.57
Total feed intake (kg)	25.85^{b}	25.95^{b}	25.89^{b}	29.64^{a}	0.42
Daily feed intake (g/d)	461.61^{b}	463.39^{b}	462.32^{b}	529.29^{a}	7.52
Feed conversion ratio	20.04^{a}	14.26^{b}	12.63^{d}	13.11^{c}	0.7

abc: Means in the same row with different superscript are significantly ($P < 0.05$) different T1 = 0 g/kg, T2 = 0.2 g/kg, T3 = 0.4 g/kg and T4 = 0.6 g/kg enzyme inclusion levels

Non-significant variation was observed in the total protozoa and pH of the ruminal fluid of sheep across the treatments (Table 4). These values (6.53-7.00) fell within the reported pH values obtained in ruminal fluid of merino sheep (Giraldo *et al*., 2009) fed with daily 12 g of exogenous fibrolytic enzyme and the range suitable for bacteria growth and activities within the rumen (Aduku 1993). Highest values of total bacteria and total fungi count were observed in sheep fed T4 while the least total bacteria and total fungi count were recorded in sheep on T1. This might be an indication that exogenous enzyme inclusion in the diet improves the population of bacteria and fungi.

More anaerobic bacteria were isolated in the rumen of Sheep fed TMR with enzyme additives (Table 5). A total eight anaerobic were isolated in the rumen of the sheep, five were isolated from sheep on T4 while three were isolated from sheep on control diet (T1). This is an indication of improved rumen environment as a result of synergy between the host community and the dietary treatments. Anaerobic bacteria are involved in the degradation process in the rumen. They are the principal agents to ferment cell wall carbohydrates. One of the common bacteria identified in the rumen of the sheep was *Pseudomonas* spp. These bacteria are cellulose hydrolyzing organism. Oyeleke and Okusanmi, (2008) harvested the bacteria from the rumen of cow, sheep, and goat as well as Lynd *et al*. (2002) who also observed these bacteria from the rumen of calves.

Bacillus spp was also isolated from the rumen of the sheep. These bacteria were also identified in the rumen of yearling calves (Yusuf, 2011). *Bacillus spp* is ubiquitous in nature; have thin cell coats and adhere tightly to the plant cell wall. *Bacillus* includes both free-living and pathogenic species. Oyeleke and Okusanmi, (2008) also harvested the bacteria from the rumen of cow, sheep, and goat likewise Lynd *et al*. (2002) also observed these bacteria from the rumen of calves. *Citrobacter* spp. of the enterobacteriaceae family is another bacteria identified in the rumen of WAD sheep fed total mixed ration with enzyme additives. They are gram-negative, facultative anaerobic bacteria that appear as rods or coccobacilli at 0.3 -1 μm in diameter and 0.6-6 μm long. *Citrobacter* spp. is motile using their peritrichous flagella. *Enteroccocus faecalis* another gram positive, non-motile, facultative anaerobic bacteria is another bacteria isolated in the rumen of the sheep,; it ferments glucose without gas production, and does not produce a catalase reaction with hydrogen peroxide.

Table 4: Rumen Microbial population of West African Dwarf sheep fed total mixed ration with enzyme additives

Parameters	T1	T2	T3	T4	SEM
Before the experiment					
Total bacteria count(×10^6 cfu/ml	2.03	2.37	2.53	2.53	0.14
Total protozoa (×10^5ml/100g)	0.63	0.53	0.70	0.62	0.12
Total fungi (×10^6 cfu/ml)	0.83	1.10	1.27	1.67	0.16
pH	7.20	7.00	6.90	6.75	0.58
At the end of the experiment					
Total bacteria count (×10^6 cfu/ml	3.10^b	3.60^b	4.67^a	4.73^a	0.24
Total protozoa (×10^5ml/100g)	0.56	0.60	0.71	0.80	0.10
Total fungi (×10^6 cfu/ml)	1.20^b	1.67^b	2.87^a	3.27^a	0.28

ab : Means in the same row with different superscript are significantly ($P < 0.05$) different T1 = 0 g/kg, T2 = 0.2 g/kg, T3 = 0.4 g/kg and T4 = 0.6 g/kg enzyme inclusion levels

Table 5: Suspected bacteria isolated from the rumen of WAD Sheep

Treatments	Suspected Bacteria							
	E. coli	Staphy-lococcus aureus	Citrobac-ter spp	Pseudo-monas spp	Proteus spp	Bacillus spp	Kleb-siella oxytoca	Entero-coccus fecalis
	Occurrence rates of bacteria isolated from the rumen of WAD sheep at the start of the study							
T1	+	+	+	+	_	-	_	_
T2	+	+	+	_	+	+	_	_
T3	+	+	+	_	+	+	+	_
T4	+	+	_	_	_	+	_	+
	Occurrence rates of bacteria isolated from the rumen of WAD sheep at the end of the study							
T1	+	_	+	_	_	_	_	_
T2	+	_	+	_	+	_	_	+
T3	+	_	_	+	+	+	_	_
T4	+	+	+	+	+	_	_	_

T1 = 0 g/kg, T2 = 0.2 g/kg, T3 = 0.4 g/kg and T4 = 0.6 g/kg enzyme inclusion levels

CONCLUSION

Enzyme additive (containing cellulase, xylanase and beta-glucanase) at 0.4 g/kg level improved the weight gain and feed intake of WAD sheep fed total mixed ration. It also increased the population of bacteria and fungi as well as the suspected bacteria isolated in the rumen of WAD sheep. It was therefore concluded that enzyme additive could be added to a total mixed ration for WAD sheep at 0.4g/kg TMR for improved feed intake and weight gain, and to increase the population of bacteria and fungi in the rumen.

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