RUMEN FERMENTATION CHARACTERISTICS OF WEST AFRICAN DWARF GOATS FED ENZYME SUPPLEMENTED TOTAL MIXED RATION IN THE DRY SEASON

ADEBAYO, Kafayat Omowumi, ADERINBOYE, Ronke Yemisi, ISAH, Olubukola Ajike and ONWUKA Christopher Friday Ijeoma
Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Nigeria.

Corresponding Author: Adebayo, K. O. Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Nigeria. **Email**: kaflak2001@yahoo.com **Phone**: +234 8035366932

ABSTRACT

The study was conducted to investigate the effect of exogenous enzyme additive on rumen environment parameters of West African Dwarf (WAD) goats fed total mixed ration (TMR) for a period of 70 days. Four experimental diets were formulated with enzyme included at 0.0, 0.2, 0.4 and 0.6 g/kg dry matter to constitute treatments 1,2, 3 and 4 respectively. Sixteen growing WAD goats were subjected to the four dietary treatments in a completely randomized design. Rumen fluid was collected from the goats before the commencement and at the end of the feeding trial. Data were collected on rumen pH, ammonia nitrogen, volatile fatty acids (VFAs) production and microbial population. Enzyme additive significantly ($p<0.05$) affect the total VFAs, propionic acid and butyric acid production with the highest values (86.23, 26.17, 14.87 Mm/100ml) for the three respectively obtained at T4(0.6g/kg). The bacteria count was significantly highest (5.20×10^6cfu/ml) at T3 although not statistically different from what was obtained at T4. A total of 5 bacteria (Bacteroides, Clostridium, Fusobacterium, Streptococcus and Peptococcus spp.), 1 fungus (Aspergillus spp.) and 2 protozoa (Holotrich spp. and Trichuris spp.) species were isolated from the rumen at the end of the study. It was therefore concluded that exogenous enzyme containing cellulase, xylanase and beta glucanase can be included in a TMR for WAD goats at 0.4g/kg for increased bacteria count and total VFAs production.

Keywords: Exogenous enzyme, Total mixed ration, Goats, Rumen fluid, Microbial population

INTRODUCTION

Cattle, sheep and goats are important sources of animal protein for Nigerian populace. They contribute to the cultural and socio-economic life of people from up north to down south. However, the productivity of these animals is usually threatened by seasonality of their major feed resource which is grass. Grass becomes dry and of low nutritive value during the dry season leading to a marked decrease in voluntary intake and digestibility. Ruminant livestock raised in this region, therefore, tend to reflect the cyclical variation in the quantity and quality of these available forages (Bamikole and Babayemi, 2008). Hence, during the dry season, ruminant feeding system is usually based on poor quality tropical foliage, crop residues or agro-industrial by-products (Oni *et al*., 2012). These are very rich in structural carbohydrates; cellulose and hemicellulose. The complex network formed by these carbohydrates and lignin reduces their digestibility and efficient utilization by ruminants. Over the years, significant improvements in forage cell wall digestibility have been achieved through forage breeding programs and agronomic advances. Despite these improvements, low forage digestibility continues to limit the intake of available energy by ruminants and correspondingly contribute to excessive nutrient excretion by livestock (Beauchemin *et al*.,

2003). Exogenous fibrolytic enzyme might enhance attachment and improve the access of microorganisms to the cell wall matrix thereby increasing the rate of digestion (Nsereko *et al*., 2000). According to Beauchemin *et al*. (2003), exogenous fibrolytic enzyme hold promise as a means of increasing forage utilization and improving the productive efficiency of ruminants. Digestion of plant cell walls in fibrolytic feeds by ruminants is possible mainly due to the activities of bacteria, protozoa and fungi. Exogenous enzymes are added to stimulate rumen digestive microorganisms' activities. The aim of this research was therefore to investigate the effect of exogenous enzyme additive on rumen environment parameters (pH, volatile fatty acid production, ammonia nitrogen and microbial population) of West African dwarf goats fed total mixed ration in the dry season.

MATERIALS AND METHODS

Experimental Site: Chemical analyses were carried out at the Laboratories of Animal Nutrition and Veterinary Microbiology Departments, while the *in vivo* study was carried out at the Small Ruminant Unit of the Teaching and Research Farms, Federal University Agriculture, Abeokuta, Ogun State. Ogun state is a derived savannah vegetation zone of south-west Nigeria. The climate in the area falls within the tropical region, with a wet season from March to October and dry season from November to February. Annual rainfall average is about 1100 mm and the peak rainfall occurs in June to September. Abeokuta is located in south western Nigeria at latitude 7 13'49'N, longitude 3 26'11'98'E and altitude 76 cm above sea level.

Test Ingredients and Experimental Diets: The test ingredient ROXAZYME G2® an exogenous fibrolytic enzyme (containing cellulase, xylanase and beta glucanase) was obtained commercially. Other feed ingredients were purchased from commercial feed mill in Abeokuta and maize stover from the university crop farm. Four total mixed rations (TMR) were formulated with enzymes included at 0, 0.2, 0.4 and 0.6 g/kg DM. The gross composition of the experimental diets (TMR) was as shown in Table 1.

Table 1: Gross composition of the experimental diets (total mixed ration)

Ingredients	Enzyme inclusion levels (g/kg DM)			
	T1(0)	T2(0.2)	T3(0.4)	T4(0.6)
Maize stover	30.00	30.00	30.00	30.00
Maize	10.00	10.00	10.00	10.00
Wheat offal	25.00	25.00	25.00	25.00
Soybean meal	10.00	10.00	10.00	10.00
Palm kernel cake	20.00	20.00	20.00	20.00
Bone meal	3.00	3.00	3.00	3.00
Common salt	1.50	1.50	1.50	1.50
***Premix**	0.50	0.50	0.50	0.50
ROXAZYME G2®	-	+	++	+++
Total	100.00	100.00	100.00	100.00

-, +, ++ and +++ represent 0, 0.2, 0.4 and 0.6 g/kg DM enzyme inclusion levels

Experimental Animals and Management: A total of 16 growing (WAD) goats were used for the experiment. The animals were housed in individual pens which was thoroughly washed and disinfected. The weight of the animals ranged from 8 to 11 kg, they were divided on weight equalization basis into four treatment groups. Each treatment groups had four replicate. Each animal formed a replicate. The experimental design was a completely randomized design. The animals were dewormed and treated against ecto-parasite before the commencement of the experiment. They were given an adjustment period of two weeks during which they were given maize stover and concentrate. They were fed 5 % of their body weight at 09.00hours everyday throughout the ten weeks (70 days) experimental period. Clean water was provided *ad-libitum*.

Chemical Analysis: The experimental diet was analyzed for its proximate composition (crude protein, crude fibre, ether extract and ash) according to AOAC (2000). The fibre fractions; neutral detergent fibre (NDF), acid detergent

fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991). Cellulose and hemicellulose were calculated as the differences between ADF and ADL, NDF and ADF respectively.

Collection and Analysis of Rumen Fluid: Before and after the experiment, 20 ml rumen fluid was collected from the rumen of the goats using suction tube into sample bottles. The rumen pH was determined immediately using a pH meter (3150 model, Jenway, UK). After collection, the rumen fluid collected was made free of coarse particles by filtration with four-layered cheese cloth and divided into three. One part was then acidified with 1ml of a 5 % (v/v) orthophosphoric acid solution and stored frozen at -20 ^{0}C till required for analysis of volatile fatty acids concentrations. Total volatile fatty acids distillate concentration was determined by titration of sample with 0.1N NaOH solution expressed as volatile fatty acid content. The method was a modified protocol that replaced conventional titration with potentiometric titration system. The concentration of NaOH solution was matched with volatile fatty acid content in the samples for all the samples (Siedlecka *et al.*, 2008). A second portion was used for the determination of rumen ammonia-nitrogen as described by Lanyasunya *et al.* (2007). The last portion was fixed with 10 % formalin solution (1:9 v/v, rumen fluid: 10 % formalin) for measuring microbial population by total direct count of bacteria, protozoa and fungal zoospores (Galyean, 1989). A further identification and isolation of the microbes was carried out using the conventional roll-tube technique (Hungate, 1969).

Statistical Analysis: All data collected was analyzed using one way analysis of variance in a completely randomized design and significant means were separated using Duncan's Multiple Range Test (SAS, 2003).

RESULTS AND DISCUSSION

The chemical composition of the experimental diets fed the animals was presented in Table 2. The dry matter and crude protein of the ration were 93.00 % and 14.95 % respectively. The dry matter concentration was adequate to support a reasonable amount of dry matter intake as dry matter is a measure of all other nutrients minus water. The crude protein content was higher than 10 – 12 % crude protein reported by Gatenby (2002) as moderate level for ruminant production. The ash content is high, an indication that the diet was rich in minerals. The fibre fractions (NDF, ADF and ADL) values were also high and this was as a result of dried materials which constituted the diets. The NDF content of 70 % in the diet was a bit higher than 60 – 65 % (600 – 650 g/kg DM) suggested as the limit above which intake of tropical feeds by ruminants would be limited (Van Soest *et al.*, 1991).

Table 2: Chemical composition of experimental diet

Parameters	Percentage (**%**)
Dry matter	93.00
Crude protein	14.95
Ether extract	9.17
Ash	16.00
Neutral detergent fibre	70.00
Acid detergent fibre	47.33
Acid detergent lignin	35.33
Cellulose	12.00
Hemicellulose	22.67

However, the fibre fractions showed that the diets have the potential to support intestinal movement and proper rumen function (Oni *et al.*, 2010). Moderate fibre levels facilitate colonization of ingesta by rumen microorganism which in turn might induce higher fermentation rates, hence improving digestibility, intake and animal growth performance (Klopfenstein *et al.*, 2001).

Table 3 showed the result of rumen pH, ammonia-nitrogen concentration, total volatile fatty acid, acetic acid, propionic acid and butyric acid contents of the rumen fluid of West African dwarf goats before the commencement and at the end of the experiment. Rumen pH values of the animals were not significantly different ($p>0.05$) from one another before feeding the experimental diets (a range of 6.60 – 6.63 pH) and after the study (a range of 6.33 – 6.44 pH).

Table 3: Rumen environment parameters of West African dwarf goats fed experimental diets

Parameters	Before the experiment				After the experiment			
	T1	T2	T3	T4	T1	T2	T3	T4
pH	6.61 ± 0.82	6.63 ± 0.05	6.60 ± 0.11	6.63 ± 0.08	6.30 ± 0.04	6.23 ± 0.08	6.28 ± 0.14	6.44 ± 0.02
Ammonia-Nitrogen (mg/100ml)	5.99 ± 0.41	6.00 ± 0.20	6.83 ± 0.41	7.07 ± 0.80	6.94 ± 0.04	6.90 ± 0.05	7.29 ± 0.08	7.23 ± 0.11
Total VFAs (mM/100ml)	52.80 ± 0.82	53.93 ± 0.41	70.10 ± 2.04	71.40 ± 0.41	52.70 ± 0.99^{d}	62.00^{c} ± 0.83^{d}	74.80 ± 0.82^{b}	86.23 ± 0.02^{a}
Acetic acid (mM/100ml)	28.40 ± 0.81	27.53 ± 0.22	33.20 ± 1.31	34.20 ± 1.01	36.90 ± 0.41	33.00 ± 1.08	41.87 ± 0.41	36.20 ± 0.71
Propionic acid (mM/100ml)	12.60 ± 0.65^{c}	15.80 ± 0.25^{b}	22.40 ± 0.41^{a}	22.00 ± 0.53^{a}	9.90 ± 0.00^{d}	18.37 ± 0.71^{c}	23.90 ± 0.41^{b}	26.17 ± 1.08^{a}
Butyric acid (mM/100ml)	9.20 ± 0.14	8.80 ± 0.41	11.50 ± 0.20	12.47 ± 0.71	5.27 ± 0.10^{c}	7.57 ± 0.23^{bc}	7.90 ± 1.08^{b}	14.87 ± 1.08^{a}

*^{a,b,c} show means within the row that are significantly different ($p < 0.05$), **T1:** 0g/kg enzyme inclusion level, **T2:** 0.2g/kg enzyme inclusion level, **T3:** 0.4g/kg enzyme inclusion level, **T4**: 0.6g/kg enzyme inclusion level*

All the values recorded fell within the reported values (6.00 – 7.20 pH) suitable for the growth and activities of microbes (Jallow and Hsia, 2011). Kamra (2005) reported a range of 6.0-6.9 pH for optimum growth of rumen bacteria. The pH of the ruminal content is probably the most important ruminal factor affecting microbial population and their activities (Nagaraja, 2012). Rumen ammonia-nitrogen concentration ranged between 5.99 – 7.07 mg/100ml in treatment 1 and 4 respectively before the study and 6.90 – 7.29 mg/100ml at the end of the study and was not significant influenced ($p>0.05$) by the experimental diets. The values were however within the range of 5 – 20 mg/100ml reported by Zareian *et al*. (2013) as suitable for ruminal microbial activities. The total volatile fatty acids (VFAs) concentration was significantly ($p<0.05$) affected by the experimental diets with the highest value (86.23 mM/100ml) obtained at T4 (0.6 g/kg DM enzyme inclusion level) and the lowest value (52.70 mM/100ml) obtained at T1 (control with no enzyme inclusion). Propionic acid and butyric acid followed the same trend. This indicated that animals fed T4 had their feed properly degraded as a result of enzyme inclusion resulting in increased VFAs production. Volatile fatty acid is the major end product of microbial degradation of fibre in the rumen. Aluwong *et al*. (2010) reported that volatile fatty acids (acetate, propionate and butyrate) are the products of the anaerobic microbial fermentation of complex carbohydrates in the fore stomach and large intestine. They provide more than 70 % of the ruminants' energy supply. In addition to this they also serve as building block of milk synthesis; acetate is a necessary component in the formation of milk fat, propionate is used for glucose production which is needed for synthesis of milk sugar (lactose) (Aluwong *et al*. 2010). Production of VFAs is generally affected by the type of and amount of plant materials as well as pH of the rumen. High roughage diets result in increased proportion of acetate whereas herbage with high levels of water-soluble carbohydrates or concentrate based diets results in increased proportion of propionate (Annison *et al*., 2002). This was evident in this study as acetic acid values were higher across the treatments than propionic acid values as the diet was high in fibre.

Rumen microbial population of West African dwarf goats fed the experimental diets was shown in Table 4. Bacteria count was significantly ($p<0.05$) affected by the dietary treatments while fungi and protozoa counts were not significantly affected. The diets increased the bacteria count from a range of 0.70 – 0.85 × 10^6 cfu/ml before the experiment to 3.20 – 5.20× 10^6 cfu/ml. The highest value (5.20 ×10^6 cfu/ml) was obtained in T3 although it was not significantly different ($p>0.05$) from the value

Table 4: Rumen Microbial population of West African dwarf goats fed experimental diets

Parameters	Before the experiment				After the experiment			
	T1	T2	T3	T4	T1	T2	T3	T4
Bacteria count (× 10^6 cfu/ml)	0.75 ± 0.04	0.70 ± 0.04	0.79 ± 0.04	0.85 ± 0.02	3.20 ± 0.45[b]	3.90 ± 0.41[b]	5.20 ± 0.08[a]	4.25 ± 0.41[ab]
Fungi count (× 10^6 cfu/ml)	0.05 ± 0.004	0.03 ±0.004	0.01 ± 0.00	0.03 ± 0.00	0.23 ± 0.04	0.67 ± 0.04	0.00 ± 0.00	0.20 ± 0.04
Protozoa count (protozoa/ml)	166.00 ± 2.45	166.67 ± 0.27	200.00 ± 20.41	200.00 ± 54.01	166.70 ± 0.27	200.00 ± 54.01	200.00±20.41	200.00 ± 20.41

[a,b,] *show means within the row that are significantly different (p < 0.05), T1: 0g/kg enzyme inclusion level, T2: 0.2g/kg enzyme inclusion level, T3: 0.4g/kg enzyme inclusion level, T4: 0.6g/kg enzyme inclusion level*

(4.25× 10^6 cfu/ml) obtained in T4. This implied that exogenous enzyme addition at 0.4 and 0.6 g/kg DM inclusion levels improved fibre degradation and consequently bacteria population by providing the required energy and protein for their growth. As they increase in number fibre degradation increases at a higher rate and invariably microbial protein available for the animals also increase. This is indicative of a normal rumen environment. Bacteria count of rumen fluid is dependent on rumen ammonia concentration and pH of rumen fluid, and both factors are dependent on type of diet. Rumen bacteria is the principal agent for fermenting plant cell wall carbohydrates hence they constitute the largest proportion of microorganism in the rumen in relation to fungi and protozoa. The bacteria, fungi and protozoa isolated in the rumen fluid of the experimental goats and their occurrence rate were presented in Tables 5, 6 and 7 respectively. The bacteria species isolated include; *Bacteroides*, *Clostridium*, *Fusobacterium*, *Streptococcus* and *Peptococcus*. *Clostridium* was isolated in the rumen fluid of all the animals used for the experiment. Bayer *et al*. (2008) reported that *Clostridium* species produce cellulosomes that are particularly designed for efficient degradation of plant cell wall polysaccharides. Bacteroides are concerned with the digestion of many carbohydrates including important polysaccharides such as cellulose, xylan and starch and appear to be concerned in protein digestion. Three species in this group: *Bacteroides succinogens*, *Bacteroides amylophilus* and *Bacteroides ruminicola* has been named (Hamlin and Hungate, 1956). According to Mould *et al*. (2005) the main rumen bacteria species are *Fibrobacter succinogens, Ruminococcus amylophilus, Prevotella ruminicola, Butyrivibrio fibrosolvens, Ruminococcus* spp. *Selenomonas ruminantum, Streptococcus bovis, Eubacterium ruminatum, Lactobacillus* spp. and *Megasphaera elsdenii.* In this study only *Streptococcus* spp. was similarly identified. *Ruminococcus flavafaciens, Ruminococcus albus, Bacteroides succinogens* and *Butyrivibrio fibrosolvens* were reported by Preston and Leng (1987) as the most common rumen bacteria for fibre degradation. Only *Bacteroides* spp. was similarly identified in this group. This may be attributed to the technique used for identification. There have been reports of limitations in the use of culture based technique to evaluate bacteria populations as it substantially underestimates the diversity of microorganism within the rumen (Fernendo *et al*., 2010). Rumen fungi have been shown to digest cellulose and xylans which shows that they may play a role in helping the ruminant host to digest plant materials (Preston and Leng, 1987). Rumen anaerobic fungi actively colonize plant cell walls and account for up to 8 – 12 % of the microbial biomass in rumen (Rezaeian *et al*., 2004). It has been reported that *Caecomyces cummunis, Piromyces cummunis and Neocallismastix frontalis* effectively take part in fibre digestion in ruminants (Dey *et al*., 2004; Lee *et al*., 2004). The rhizoids of their vegetative thalli penetrate deep into plant tissues better than bacteria and protozoa, and thus achieve access to plant materials otherwise unavailable to other rumen microorganisms.

Table 5: Occurrence rates of bacteria isolated from the rumen of West African dwarf goats fed experimental diets

Treatments	Before the study					
	***Bacteroides* spp.**	***Clostridium* spp.**	***Fusobacterium* spp.**	***Streptococcus* spp.**	***Pseudomonas* spp.**	***Peptococcus* spp.**
T1	-	+	-	-	-	-
T2	+	+	+	-	+	-
T3	+	+	+	+	+	-
T4	-	+	-	-	+	-
	At the end of the study					
T1	+	+	+	+	-	+
T2	+	+	-	-	-	+
T3	+	+	-	-	-	+
T4	-	+	-	+	-	-

T1: 0g/kg enzyme inclusion level, T2: 0.2g/kg enzyme inclusion level, T3: 0.4g/kg enzyme inclusion level, T4: 0.6g/kg enzyme inclusion level, +: present, -: absent

Table 6: Occurrence rates of fungi isolated from the rumen of West African dwarf goats fed experimental diets

Treatments	Before the study				
	Caecomyces cummunis	***Piromyces communis***	***Aspergillus* spp.**	***Neocallismastix frontalis***	***Yeast***
T1	-	-	-	-	-
T2	-	-	-	-	-
T3	-	-	-	-	-
T4	-	-	-	-	-
	At the end of the study				
T1	-	-	+	-	-
T2	-	-	-	-	-
T3	-	-	-	-	-
T4	-	-	-	-	-

T1: 0g/kg enzyme inclusion level, T2: 0.2g/kg enzyme inclusion level, T3: 0.4g/kg enzyme inclusion level, T4: 0.6g/kg enzyme inclusion level, +: present, -: absent

Table 7: Occurrence rates of protozoa isolated from the rumen of West African dwarf goats fed experimental diets

Treatments	Before the study			
	***Holotrich* spp.**	***Epidinium* spp.**	***Entodinium* spp.**	***Trichuris* spp.**
T1	-	-	-	-
T2	+	-	-	+
T3	+	-	-	+
T4	-	-	-	-
	At the end of the study			
T1	+	-	-	-
T2	+	-	-	+
T3	+	-	-	+
T4	+	-	-	-

T1: 0g/kg enzyme inclusion level, T2: 0.2g/kg enzyme inclusion level, T3: 0.4g/kg enzyme inclusion level, T4: 0.6g/kg enzyme inclusion level, +: present, -: absent

This infiltration leads to a more rapid degradation of forage entering the rumen (Nagpal *et al*., 2009). These fungi secrete high levels of very active fibre-degrading enzymes (cellulases, hemicellulases, xylanases, avicelases, glycosidases etc.) found to be associated with rhizomycelia (Williams *et al*., 1994; Lee *et al*., 2001). However, only one fungus (*Aspergillus* sp.) was identified at the end of this study and it does not include any of the aforementioned fungi. This may also be as a result of the technique used in identification. The dietary treatments did not increase the protozoa number which is an advantage. Two protozoa species were isolated; *Holotrich* spp. and *Trichuris* spp. Protozoa are now recognized as having an overall negative effect on the rumen particularly where ruminants are fed forage diets low in true protein (Bird *et al*., 1990). Protozoa ingest and digest bacteria and reduce the bacteria biomass in the rumen (Coleman, 1975) and consequently the protein supply to the animals.

Conclusion: Exogenous enzyme inclusion in a total mixed ration for West African dwarf goats (WAD) increased total volatile fatty acids (VFAs) production and bacteria count similarly at 0.4 g/kg DM and 0.6 g/kg DM levels of inclusion. Therefore, for increased VFAs production and bacteria count, exogenous enzyme used in this study can be included in a total mixed ration for WAD goats at 0.4 g/kg DM level of inclusion. It was however noted in this study that despite being on the same diet (only enzyme levels vary) the animals did not have uniform species of bacteria, fungi and protozoa in their rumen fluid.

REFERENCES

ALUWONG, T., KOBO, P. I. and ABDULLAHI, A. (2010). Volatile fatty acids production in ruminants and the role of monocarboxylate transporters: a review. *African Journal of Biotechnology*, 9(38): 6229 – 6232.

ANNISON, E. F., LINDSAY, D. B. and NOLAN, J. V. (2002). Digestion and metabolism. Pages 95 – 118. *In*: FREER, M. and DOVE, H. (Eds.). *Sheep Nutrition*. CABI, Wallingford, UK.

AOAC (2000). *Official Methods of Analysis*. 17th Edition, Association of Official Analytical Chemists, Washington DC, USA.

BAMIKOLE, M. A. and BABAYEMI, O. J. (2008). Chemical composition and in sacco dry matter degradability of residue and by-products of palm fruit processing in the rumen of steers. *Animal Science Journal*, 79(3): 314 – 321.

BAYER, E. A., LAMED, R., WHITE, B. A. and FLINT, H. J. (2008). From cellulosomes to cellulosomics. *The Chemical Record*, 8(6): 364 – 377.

BEAUCHEMIN, K. A., COLOMBATTO, D., MORGAVI, D. P. and YANG, W. Z. (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science*, 81(14 Suppl 2): E37 – E47.

BIRD, S. H., NOLAN, J. V. and LENG, R. A. (1990). The nutritional significance of rumen protozoa. Pages 151 – 160. *In*: HOSHINO, S., ONODERA, R., MINATO, H. and ITIBASHI, H. (Eds.). *The Rumen Ecosystem: The Microbial Metabolism and its Regulation*. Springer-Verlag, Tokyo.

COLEMAN, G.S. (1975). Interrelationship between rumen ciliate protozoa and bacteria. *In*: MCDONALD, I. W. and WARNER, A.C.I. (Eds.). *Digestion and Metabolism in the Ruminant*. University of New England, Armidale, Australia.

DEY, A., SEHGAL, J. P., PUNIYA, A. K. and SINGH, K. (2004). Influence of an anaerobic fungal culture (*Orpinomyces* sp.) administration on growth rate, ruminal fermentation and nutrient digestion in calves. *Asian Australasian Journal of Animal Sciences*, 17(6): 820 – 824.

FERNANDO, S. C., PURVIS, H. T., NAJAR, F. Z., SUKHARNIKOV, L. O., KREHBIEL, C. R., NAGARAJA, T.G., ROE, B. A. and DESILVA, U. (2010). Rumen microbial population dynamics during adaptation to a high-grain diet. *Applied and*

Environmental Microbiology, 76(22): 7482 – 7490.

GALYEAN, M. (1989). *Laboratory Procedure in Animal Nutrition Research*. Department of Animal and Life Science, New Mexico State University, USA.

GATENBY, R.M. (2002). *Sheep.* Revised Edition, Tropical Agricultural Series, MacMillan Publishers Limited, 6700AD Wageningen, The Netherlands.

HAMLIN, L. J. and HUNGATE, R. E. (1956). Culture and physiology of a starch-digesting bacterium (*Bacteroides amylophilus* n. sp.) from the bovine rumen. *Journal of Bacteriology*, 72(4): 548.

HUNGATE, R. E. (1969). A roll-tube method for cultivation of strict anaerobes. Pages 117 – 132. *In*: NORRIS, J. R. and RIBBONS, D. W. (Eds.). *Methods in Microbiology*. Academic Press, London.

JALLOW, D. B. and HSIA, L .C. (2011). Effect of six feed supplement on ruminal degradation characteristics and amino acid profile of sheep. *International Journal of Animal and Veterinary advances,* 3(5): 367-373.

KAMRA, D. N. (2005). Rumen microbial ecosystem. *Current Science*, 89(1): 124 – 135.

KLOPFENSTEIN, T. J., MASS, R. A., CREIGHTON, K. W. and PATTERN, H. H. (2001). Estimating forage and protein degradation in the rumen. *Journal of Animal Science*, 79 (E Suppl.): 208 – 217.

LANYASUNYA, T. P., WANG, H., KARIUKI, S. T., MUKISIRA, E. A., ABDULRAZAK, S. A., KIBITOK, N. K. and ONDIEK, J. O. (2008). The potential of *Commelina benghalensis* as a forage for ruminants. *Animal Feed Science and Technology*, 144(3): 185 – 195.

LEE, S. S., HA, J. K. and CHENG, K. J. (2001). Effects of LCFA on the gas production, cellulose digestion and cellulase activities by the rumen anaerobic fungus, *Neocallimastix frontalis* RE1. *Asian-Australasian Journal of Animal Sciences*, 14(8): 1110 – 1117.

LEE, S. S., CHOI, C. K., AHN, B. H., MOON, Y. H., KIM, C. H. and HA, J. K. (2004). *In vitro* stimulation of rumen microbial fermentation by a rumen anaerobic fungal culture. *Animal Feed Science and Technology*, 115(3): 215 – 226.

MOULD, F. L., KLIEM, K. E., MORGAN, R. and MAURICIO, R. M. (2005). *In vitro* microbial inoculum: a review of its function and properties. *Animal Feed Science and Technology*, 123: 31 – 50.

NAGARAJA, T. (2012). A microbiologist's view on improving nutrient utilization in ruminants. Pages 149 – 175.*In*: *23rd Annual Ruminant Nutrition Symposium.* Department of Animal Sciences, University of Florida, Gainesville, Florida.

NAGPAL, R., PUNIYA, A. K., GRIFFITH, G., GOEL, G., PUNIYA, M., SEHGAL, J. P. and Singh K. (2009). Anaerobic rumen fungi: potential and applications. *Agriculturally Important Microorganisms*, 1: 375 – 393.

NSEREKO, V. L., MORGAVI, D. P., RODE, L. M., BEAUCHEMIN, K. A. and MCALLISTER, T. A. (2000). Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfalfa hay fiber by mixed rumen microorganisms in vitro. *Animal Feed Science and Technology*, 88(3): 153 – 170.

ONI, A. O., ARIGBEDE, O. M., ONI, O. O., ONWUKA, C. F. I., ANELE, U. Y., ODUGUWA, B. O. and YUSUF, K. O. (2010). Effects of feeding different levels of dried cassava leaves (*Manihot esculenta,* Crantz) based concentrates with *Panicum maximum* basal on the performance of growing West African dwarf goats. *Livestock Science*, 129(1): 24 – 30.

ONI, A. O., ARIGBEDE, O. M., SOWANDE, O. S., ANELE, U. Y., ONI, O. O., ONWUKA, C. F. I., ONIFADE, O. S., YUSUF, K. O., DELE, P. A. and ADERINBOYE, R. Y. (2012). Haematological and serum biochemical parameters of West African dwarf goats fed dried cassava leaves-based concentrate diets. *Tropical*

Animal Health and Production, 44: 483 – 490.

PRESTON, T. R. and LENG, R. A. (1987). *Matching Ruminant Production Systems with Available Resources in the Tropics and Sub-Tropics*. Penambul Books, Armidale, Australia.

REZAEIAN, M., BEAKES, G. W. and PARKER, D. S. (2004).Distribution and estimation of anaerobic zoosporic fungi along the digestive tracts of sheep. *Mycological Research*, 108(10): 1227 – 1233.

SIEDLECKA, E. M., KUMIRSKA, J., OSSOWSKI, T., GLAMOWSKI, P., GOLEBIOWSKI, M., GAJDUS, J., KACZYNSKI, Z. and STEPNOWSKI, P. (2008).Determination of volatile fatty acids in environmental aqueous samples. *Polish Journal of Environmental Studies,* 17(3): 351 – 356.

SAS (2003). *The SAS System for Windows*. Release 9.1, Statistical Analysis Systems (SAS) Institute, Cary, North Carolina, USA.

VAN SOEST, P. J., ROBERTSON, J. B. and LEWIS, B. A.(1991).Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides determination in relation to animal nutrition. *Journal of Dairy Science,* 74: 3583 – 3597.

WILLIAMS, A.G., WITHERS, S.E., NAYLOR, G.E. and JOBLIN, K.N.(1994). Effect of heterotrophic ruminal bacteria on xylan metabolism by the anaerobic fungus *Piromyces communis*. *Letters in Applied Microbiology,*19(2): 105 – 109.

ZAREIAN, M., EBRAHIMPOUR, A., MOHAMED, A. K. S. and SAARI, N. (2013). Modeling of glutamic acid production by *Lactobacillus plantarum* MNZ *Electronic Journal of Biotechnology* 16(4): doi:10.2225/vol16-issue4-fulltext-10