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The effect of ruminal incubation of bioactive yeast (*Saccharomyces cerevisiae*) on potential rumen degradability of *Panicum maximum* and *Centrosema pubescens* in West African dwarf sheep

LO Aka[1*], NC Ugochukwu[1], A Ahmed[2] & NN Pilau[3]

[1] *Department of Veterinary Physiology &Pharmacology*
University of Nigeria, Nsukka.
[2]*National Veterinary Research Institute, Zonal Laboratory Birnin Kebbi,*
Kebbi State.
[3]*Department of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto*

*Correspondence: Tel.: + 2348134576173, E-mail: Lawrence_aka@yahoo.com

Abstract
The rising interest in the use of organic and inorganic substances in manipulating rumen function for improved fermentative activity has provided avenues for the inclusion of various species of yeast cultures in ruminant diets. In this study, we investigated the effect of bioactive yeast (*Saccharomyces cerevisiae)*, on rumen degradative function of the West African Dwarf Sheep (WADS) in terms of fermentable organic matter, crude protein and crude fiber of *Panicum maximum* and *Centrosema pubescens*. Three inclusion levels of *Saccharomyces cerevisiae*, 200, 500 and 800 milligrams were infused into the rumen of three groups (A, B and C), of three WAD sheep each. Another group (D) of same animal number served as the control. *In vivo* rumen potential degradability studies, using the nylon bag technique was performed using *Panicum maximum* and *Centrosema pubescens* in all the groups. The result of the study showed that bioactive yeast improved the potential rumen degradability of crude protein, crude fibre and organic matter fractions of *Panicum maximum* and *Centrosema pubescens* in a rather dose dependent manner compared to the control. There was a significant correlation (r = 0.56) between degradability, dose and time of incubation for crude fibre and organic matter fractions but not for crude protein. These observations suggest that regulated dietary inclusion of bioactive yeast can be used to bioengineer the rumen towards efficient fibre breakdown, particularly forages of poor protein quality and high fibre content, for efficient production of volatile fatty acids as well as probably enhancing other aspects of rumen functions.

Keywords: Bioactive Yeast, Degradability, Forages, Rumen, WADS.

Introduction

Nutrition is one of the most important factors affecting animal production in most part of the world. For ruminants, this is particularly more pronounced in the tropics where forages are more likely to be of poor protein quality and high fiber content (Devendra, 1988). With the high cost and seasonality of feeds, livestock farmers have been stimulated to search for alternative feed resources that can economically supplement the conventional feed ingredients in rations without adverse effects on the health and performance of animals (Lufadeju & Olorunju, 1986; Smith, 1988).

Ruminants have a unique ability to convert feedstuff of low nutritional value or unfit for human consumption into useful end products that are utilised for productivity and growth. This is possible because of microbial fermentation taking place in the rumen and reticulum (Egbo, 2008). For optimal utilization of feed, it would be desirable to be able to quantify and express, in feeding standard, the needs for rumen micro-organisms and for the host animal separately (Czerkawski, 1986).

Aside from the natural capacities of the rumen microbes to degrade fibrous feedstuffs and express their nutrient both to the host and the microbes themselves, several organic and inorganic compounds have been used to engineer the activities of the microbes and improve rumen function generally (Dawson & Newman, 1987).

One of such organic compounds that has been popularly used *in vitro* via inclusion in the diet of ruminant animals is yeast (*Saccharomyces cerevisiae*). The use of yeast in animal nutrition has been the subject of numerous studies over the last decade. It has been postulated that its inclusion in the diet of farm animals is beneficial; however the right amount must be added for optimal results to be obtained (Fickers, *et al.*, 2005). A lot of research has been carried out in the developed world, with some works studying its effects on the rumen fauna, protein and cellulose digestion, rumen development, growth rate and general performance etc, whereas other works studied the efficacy of the different species of yeast and even the different strains of each

species (Martins *et al*., 1984; Dawson & Newman, 1987; Williams *et al*., 1991; Newman & Spring, 1993; Fickers, *et al*., 2005). Some of the commonly used yeast in farm animal diet include *saccharomyces cerevisiae, Aspergillus oryzae* etc.

Most of these earlier studies were centred on exotic breeds; studies using bioactive yeast in tropical ruminants are relatively new. In the tropics, the use of yeast in animal nutrition and hence research involving yeast has been directed mainly on poultry (Muhammad, *et al*., 2008).), with few if any, focusing on other aspects of animal husbandry particularly the ruminants. In order to evaluate the possible impact of yeast on ruminant nutrition, this study was to determine the effect of ruminal incubation of bioactive yeast *(Sacharomyces cerevisiae)* on the rumen degradability of crude protein, crude fibre and organic matter fractions of *Panicum maximum* and *Centrosema pubescens* in West African dwarf sheep using the nylon bag technique. The result of which would be valuable in assessing the bioengineering efficacy of yeast on the efficiency of rumen fermentative activity.

Materials and Methods

Animal

Eight male adult sheep of average body weight 16.93±3.44 kg purchased from Ibagwa market in Nsukka Local Government Area of Enugu State, southern Nigeria were used for this study. They were quarantined for 14 days and acclimatised for another 21 days after purchase at the small animal unit of the Department of Veterinary Physiology/Pharmacology, University of Nigeria, Nsukka. They had an average body weight of 17.2 ±3.66 kg after acclimatisation. Ectoparasites and endoparasites were routinely controlled together with prescribed vaccination. The sheep were identified using tag plates. Nylon bags of polyamide cloth with a pore size of 36 microns and an aperture area of 30% were used. The bags measured 7.5x10cm each and were all soaked in 70% ethanol for 24 hours before use. Each nylon bag was identified using a white nylon thread bearing a plastic plate at one end and firmly attached to the nylon bag at another end. The identification was done in such a way as to reflect the sample contained in the nylon bag, the rumen incubation time and the animal into which the bag is incubated. All the eight sheep were rumen fistulated as described by Santra & Karim (2002).

Feeding

During the quarantined period, the animals were fed with freshly harvested *Panicum maximum* and *Centrosema pubescens* together with water ad libitum.

Forage samples: *Panicum maximum* (Guinea grass) and *Centrosema pubescens* were harvested and identified using a specimen sample at the herbarium of the Department of Botany, University Of Nigeria, Nsukka. They were dried and milled to pass through 1mm sieve.

Determination of immediate soluble fraction: Three grams of each ground forage sample was placed into duplicates of five nylon bags such that 20 nylon bags were used; five duplicate bags for each plant species. The nylon bags prepared as described by Orskov *et al*., (1980) were used. The feed samples contained nylon bags were suspended in a water bath maintained at 37^0C for 1 hour. At the end of the one hour, they were washed under running tap until the water was colourless. They were later dried at constant weight at 60^0C and analysed for protein content, organic matter and crude fibre contents (AOAC, 1990). The differences in the crude protein, crude fiber and organic matter of the forages before suspension and heating in water bath and after heating in a water bath and washed to clean rinse, represents the immediate soluble fraction 'a' of the forage fractions (crude protein, organic matter and crude fibre) (Aregbede *et al*., 2002).

Determination of water insoluble rumen degradable or slowly degradable feed fractions ('b' fraction): Three grams of each ground forage sample was placed in 2 nylon bags. The bags were tied in such a way that the main body of the bag was free and spread, to provide enough area for rumen liquor to flow across the nylon bag while incubated in the rumen and to enable rumen microbes come in good contact with forage samples contained in the incubated nylon bags. In randomized design of two blocks, each feed sample was incubated in three replicates per sheep for time intervals of 4, 8 and 24 hours. At the end of each incubation time each replicate sample was removed and washed thoroughly under running water until the water became colourless. They were then dried to constant weight at 60^oC using an air oven. The CP, CF and OM fractions of the dried sample were then determined (AOAC, 1990).

Determination of rumen degradability fractions

Differences in organic matter, crude protein and crude fibres before and after incubation were equivalent to forage fraction degraded in the rumen. The difference in weight of dry matter in nylon bags before and after rumen incubation represents dry matter degradability in the rumen. Also the difference between dry matter and ash before and after incubation represents the organic matter degradability. Potential rumen degradability was of all the feed fractions (CF, CP and OM) was determined.

Statistical analysis

The mean percentage rumen degradability of each feed fraction at different doses of bioactive yeast for a given incubation period of each forage were compared statistically using the least significant difference (LSD) of mean comparison. Correlation analysis of the interaction between degradability, dose of bioactive yeast and time of incubation for each forage was performed (Steel & Torrie, 1980).

Results

The results of the ruminal disappearance of the feed fractions at different periods of incubation are presented in percentages in figures 1-9. Table 1 shows the chemical composition (%) of the forages used for degradability studies.

Figure 1 represents the quantity of crude protein lost after 4hours incubation. At 0.2, 0.5 and 0.8g of yeast 5.6, 6.3 and 5.7 % respectively were the mean crude protein disappearance values compared to a control value of 5.29 % for *P. maximum*. This shows a higher CP ruminal disappearance at 0.5 g of yeast. For *Centrosema pubescens,* at 0.2, 0.5 and 0.8g of yeast 5.7, 9.6 and 6.89 respectively were lost compared to a control value of 3.6% .This showed a higher CP disappearance at 0.5 g of yeast for both forages at 4 hrs post incubation.

Figure 2 represents the quantity of crude protein lost after 8hour incubation. At 0.2, 0.5 and 0.8g of yeast 6.3, 6.5 and, 6.0 % respectively were the mean disappearance values compared to a control value of 5.8 % for *P. maximum*. For *Centrosema pubescens,* at 0.2, 0.5 and 0.8g of yeast, 10.5, 12.3 and 9.0% respectively were lost compared to a control value of 7.7 %. At 8 hrs incubation CP disappearance was observed at 0.5 g of yeast for both forages.

Figure 3 represents the quantity of crude protein lost after 12hours incubation. At 0, 0.5 and 0.8g of yeast, 9.1, 8.9 and 8.6 % were lost respectively compared to a control value of 8.46% for *P.maximum.* For *C.pubescens*, at 0.2, 0.5 and 0.8g of yeast, 11.7, 11.7 and 10.10% were lost respectively compared to a control value of 9.8%. At 12 hrs incubation there was no difference in the CP disappearance between the yeast treatments for *P. maximum.* However, for *C. pubescencs* 0.2 and 0.5 g of yeast had significantly higher CP disappearance compared to 0.8 g of yeast and the control.

Figure 4 represents the quantity of crude fibre lost after 4hours incubation. At 0.2, 0.5 and 0.8g of yeast, 6.7, 5.8 and 9.8% were lost respectively compared to a control value of 6.19% for *Panicum maximum.* For *Centrosema pubescens*, at 0.2, 0.5 and 0.8 g of yeast, 21.5, 16.5 and 17.9% respectively were lost compared to the control value of 13.76%. For *P. maximum*, the percentage disappearance of CF was not significantly different between all yeast treatments. On the contrary for *C. pubescens*, there was significantly higher percentage disappearance of CF at 0.2 and 0.5g yeast compared to the 0.8g treatment and the control.

Figure 5 represents the quantity of crude fibre lost after 8hours incubation. At 0.2, 0.5 and 0.8g of yeast, 11.6, 15.0 and 18.2 % were lost respectively, compared to a control value of 12.71% for *P. maximum*. For *C. pubescens*, at 0.2, 0.5 and 0.8g of yeast, 30.6, 23.3and 28.3% were lost respectively compared to a control value of 22.3%. This shows that at 8 hr incubation, the CF disappearance for C. pubescens was higher than P. maximum at all yeast treatment levels. The CF disappearance for C pubescens was highest at 0.2g treatment while highest CF disappearance occurred at 0.8 g treatment.

Figure 6 represents the quantity of crude fibre lost after 12hours incubation. At 0.2, 0.5 and 0.8g of yeast, 18.7, 18.7 and 21.6% were lost respectively compared to a control value of 16.6% *P. maximum*. For *C* .pubescens, at 0.2, 0.5 and 0.8g of yeast, 34.9, 33.6 and 34.6% were lost respectively compared to a control value of 29.8%. At this period of incubation therefore, there was no difference in the CF disappearance of both forages at all treatments.

Figure 7 represents the quantity of organic matter lost after 4hours incubation. At 0.2, 0.5 and 0.8 g of yeast, 18.7, 18.7 and, 21.6% were lost respectively compared to a control value of 16.6 % for *P.maximum*. For *C. pubescens,* at 0.2, 0.5 and 0.8g of yeast, 34.9, 33.6 and, 34.6% were lost respectively compared to a control value of 29.83%. For both forages, percentage disappearance of organic matter was directly related to dose of yeast with 0.8 g of yeast recording the highest organic matter disappearance.

Figure 8 represents the quantity of organic matter lost after 8hours incubation. At 0.2, 0.5 and 0.8 g of yeast, 24.7, 31.7 and 42.6% were lost respectively compared to a control value of 23.5% *P. maximum*. For *C. pubescens*, at 0.2, 0.5 and 0.8g of yeast, 34.9, 43.64, 57.4 % were lost respectively compared to a control value of and 33.8%. At this incubation period, the same pattern of disappearance, as observed at 4 hr incubation was observed. It was though reduced compared to 4 hr incubation.

Figure 9 shows the quantity of organic matter lost after 12hours incubation. At 0.2, 0.5 and 0.8g of yeast, 42.6, 51.7 and 68.6% were lost respectively compared to a control value of 33.1% for *P. maximum* For *C. pubescens*, at 0.2, 0.5 and, 0.8g of yeast, 49.5, 66.6 and 83.4% were lost respectively compared to a control value of 37.6%. A dose dependent effect as in 4 and 8 hrs incubation periods was also observed. However, at this period greatest percentage compared to those of 4 and 8 hrs incubation were recorded in each treatment.

Table 1: Chemical composition of Panicum maximum and Centrosema pubescens

Forage	Crude protein	Ash	Fibre	Fat	Moisture
C. pubescens	27.80	8.15	47.40	1.30	2.10
P. maximum	14.71	8.35	59.03	0.30	2.40

From the data above, crude protein composition of *C. pubescens* (27.80%) was higher than *P. maximum* (14.71%); On the other hand the crude fiber, composition of P. maximum (59.03%) was higher than that of *C. pubescens* (47.400. The ash content of the plants were 8.15% and 8.35% for *C.pubescens* and *P.maximum* respectively. The moisture contents for both plants were 2.10% for *C. pubescens* and 2.40% for *P. maximum*.

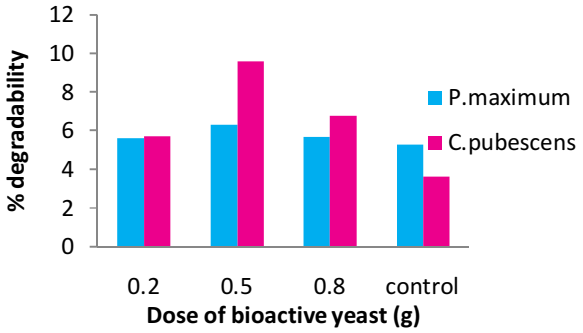

Figure 1:Percentage ruminal disappearance of crude protein in *P. maximum* and *C. pubescens* at 4 hrs incubation in WAD sheep.

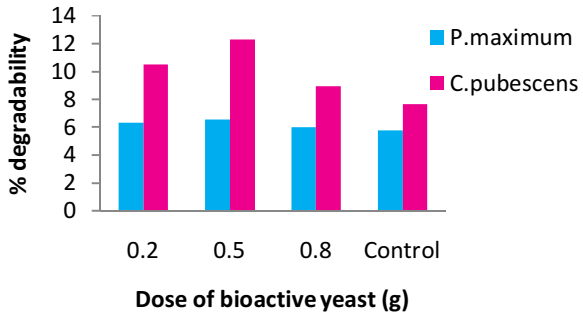

Figure 2: Percentage ruminal disappearance of crude protein in *P. maximum* and *C. pubescens* at 8 hrs incubation in WAD sheep.

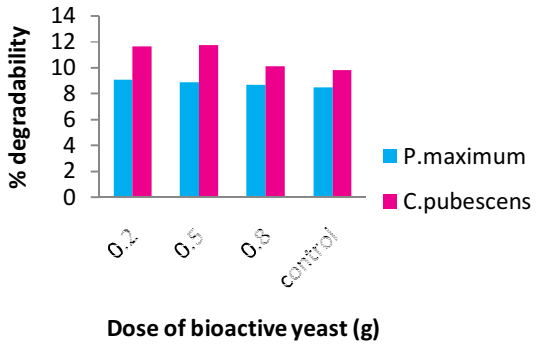

Figure 3: Percentage ruminal disappearance of crude protein in *P. maximum* and *C. pubescens* at 12 hrs incubation in WAD sheep.

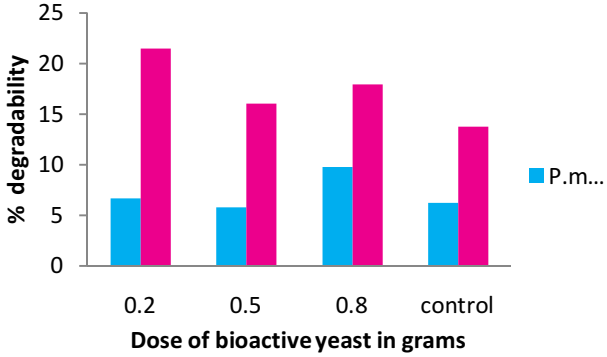

Figure 4: Percentage ruminal disappearance of crude fiber in *P. maximum* and *C. pubescens* at 4 hrs incubation in WAD sheep.

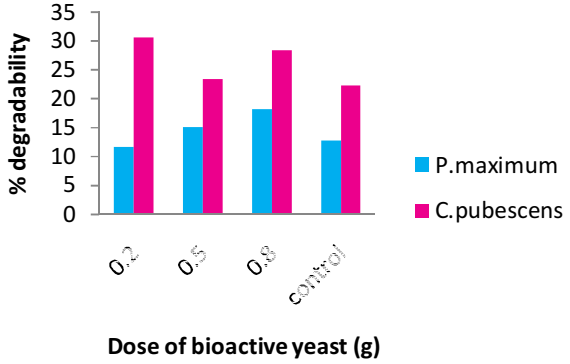

Figure 5: Percentage ruminal disappearance of crude fiber in *P. maximum* and *C. pubescens* at 8 hrs incubation in WAD sheep.

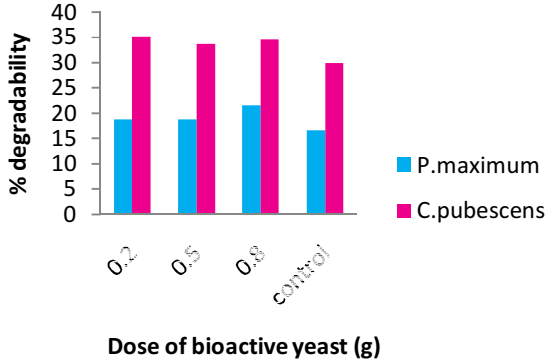

Figure 6: Percentage ruminal disappearance of crude fiber in *P. maximum* and *C. pubescens* at 12 hrs incubation in WAD sheep.

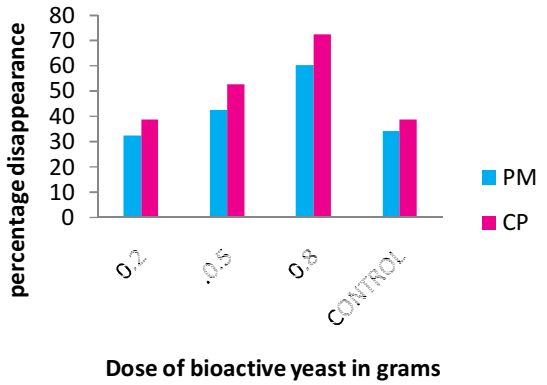

Figure 7: Organic matter potential rumen degradability of *P. maximum* and *C. pubescens* following *in vivo* bioactive yeast at 4-hour ruminal incubation.

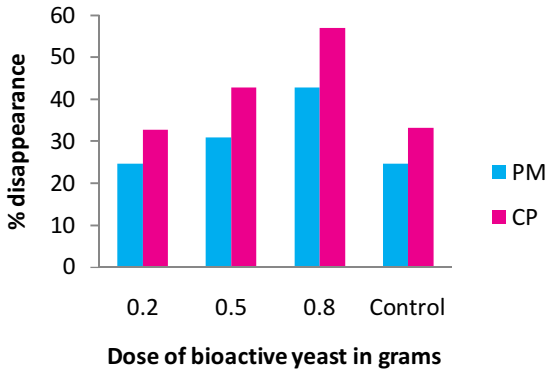

Figure 8: Organic matter potential rumen degradability of *P. maximum* and *C. pubescens* following *in vivo* bioactive yeast at 8hour ruminal incubation.

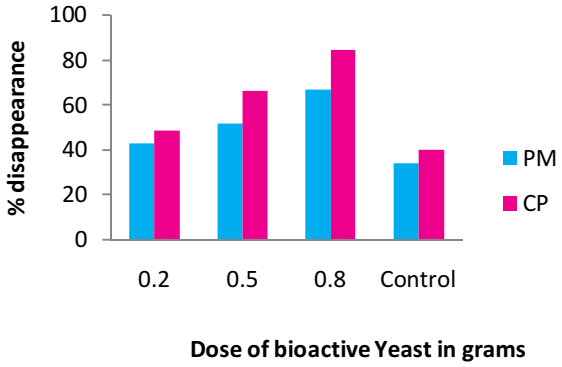

Figure 9: Organic matter potential rumen degradability of *P. maximum* and *C. pubescens* following in vivo bioactive yeast at 12 hours ruminal incubation.

Discussion

Legumes and grasses are the main sources of nutrients for ruminants, particularly because they conserve nutrients for long periods of time especially during harsh and unfavourable times such as winter and the harmattan (dry) season when resources are in short supply (Minson, 1990), as well as their high rumen degradability potential (Aka, 2004). As a result, they supply nutrients mainly to rumen microbes more than they do to the host animal. In other to improve nutrient availability to the host animal from them they are usually supplemented with other slowly degradable feeds (ARC, 1980).

In this study, the result of the proximate analysis (table 1) of the forages indicated that *C.pubescens*, a legume had a higher crude protein value 27.80% compare to that of *Panicum maximum* with a crude protein value of 14 .71 %. This finding agrees with earlier report of Morrison (1990); Aka (2004), who reported that the average crude protein content of legumes and legume-containing pastures were higher than those of grasses alone and pastures without legumes. It is particularly important to note that these values fell within the recommended 15-22 % protein level in ruminant diets (ARC, 1980). Variations in organic matter constituents of forages could be due to factors ranging from the plant part harvested, frequency of cutting, climate, stage of the plant growth, etc. On the other hand, the crude fiber content of *Panicum* maximum which was 47.40 % of the dry matter was higher than that of *Centrosema pubescens* which had a value of 22.64% of dry matter. There is usually a inverse relationship between the crude protein and crude fibre content in a given forage species and this has been revalidated in this study. The ash content of *P. maximum* was 8.35% of dry matter while that of *C. pubescens* was 8.15 % indicating a close relationship of organic matter contents of the forages studied. This observation is however in agreement with the assertion that the difference in organic matter contents of legumes and grasses generally do not exceed 5% (Ademosun, 1973). For the ether extract, *C. pubescens* had a higher value of 1.3 % compare to *P. Maximum* with a value of 0.30 % of dry matter. Minson (1990), had earlier pointed out that legumes generally have higher ether extract than grasses particularly those grown in the same area and are of same age and harvested at the same season.

Table 2 shows the immediate soluble fraction of *C. pubescens* and *Panicum maximum*. Here, it was observed that the immediate soluble fraction 'a' for *C.*

Pubescens was slightly higher than that of *P. maximum*. This implies than the water solubility of the organic and inorganic constituents of *C. pubescens* was higher than that of *P. Maximum* under the same temperature. One would expect the reverse due to higher ether level in C. pubescens but it has been demonstrated in several digestive studied that solubility of forages in water within few minutes to an hour *in vitro* is usually a function of the crude protein level but not ether level (Devendra, 1986). As a result, solubility of protein in forages is generally greater in legumes and young plants than in grasses but it decreases with age (Devendra, 1986).

The degradability characteristics of the forage fractions following the ruminal addition of bioactive yeast show that crude protein disappearance was consistently high at 0.05g of yeast at all periods of incubation. At this level (0.05g) also, the percentage disappearance at 4, 8 and 12 hours were significantly ($p<0.05$) higher than the control. This probably suggests that beyond this level, mechanisms that support increased degradability of crude protein were inhibited or static. (Figure 1-3). This observation was however different from that of crude fibre degradability. In this case, at 4 and 8 hours incubation, there were significant disappearances of crude fibre at 0.2 and 0.8 g of yeast inclusion levels compared to the control. There was no significant difference in the crude fibre disappearances between 0.5g yeast inclusion level and the control at all incubation periods. At 12 hour incubation, there was no significant difference in crude fibre degradability between all treatment levels and the control (Figure. 4-6). The organic matter degradability was however dependent on both dose of yeast as well as length of incubation for both forages. It was observed that as the dose and length of incubation increased, the degree of degradation also increased (Figure. 7-9).

In other studies digestibility of DM, NDF, ADF, OM, CP ,hemicelluloses and cellulose improved by yeast supplementation. Yeast cultures may provide factors stimulatory to rumen cellulolytic and proteolytic bacteria, *especially* when high concentrate (> *50%)* diets *are* fed (Newbold *et al*., 1995). Feeding yeast cultures has increased numbers of cellulolytic rumen bacteria (Newbold *et al*., 1995) and resulted in improved fiber digestibility (Weidmer *et al*., 1987) and improvement in digestibility of a specific nutrient with no change in total tract DM digestibility when yeast culture was fed has been reported previously (Weidmer *et al* 1987). Williams *et al*., (1991) suggested that yeast cultures may increase ruminal digestion. An increase in degradation of cellulose by pure culture of *Fibrobacter succinogenes* (Dawson, 1990) and in the rumen (Williams *et al.,* 1991) was observed after yeast addition. In sheep fed a diet containing 50% barley, Henics & Gombos (1991) observed that the activities of polysaccharide depolymerases (CMCase and xylanase) measured on solid-adherent bacteria and liquid-associated bacteria were stimulated by living yeast cells. As a consequence, the rumen degradability of cellulosic fraction of cornstalk has been significantly improved by addition of *S. cerevisiae* in the diet of lactating dairy cows or in buffalo calves fed a roughage diet (Kumar *et al.,* 1997). The positive effect of *S. cerevisiae* on plant cell wall digestion occurs at the initial stage of cellulolysis (Adams *et al*., 1981). Sometimes, digestibility of cell wall fractioning the whole digestive tract was improved by a very large extent (+ 12%) by yeasts (Fiems *et al*., 1993). Fiems *et al*., (1993) confirmed the stabilization of the microbial ecosystem by yeasts when animals are fed a diet rich in cereals. Addition of *S. cerevisiae* in the diet of sheep did not alter the degradation of feed proteins in the rumen nor the ammonia (NH_3) up-take by bacteria. It induced a large accumulation of non amino nitrogen (NAN) in the rumen due probably to a continuous release of proteins or peptides in the medium. *In situ* degradation of nitrogen (N) from soybean meal measured in fistulated lactating dairy cows was significantly increased by yeasts during the first ten hours of the kinetics. The effects of *S. cerevisiae* on volatile fatty acid (VFA) concentration are generally weak and non significant. The effect of yeasts on VFA production is diet-dependant. Using three levels of concentrate in the diet, they observed that yeasts increased VFAs only with the diet richest in concentrate. Propionogenesis is often improved by yeast culture (Erasmus *et al.,* 1992), but sometimes acetogenesis is stimulated (Chaucheyras *et al*., 1995).

Increased degradability may be due to the degradation activity of *S. cerevisiae* in addition to that of cellulolytic anaerobes in the rumen. Malcon & Kiesling (1986) showed that yeast attach to particulate fraction in the rumen and interact with other microbes in a microstructure called “microbial consortium”. Yeast decreases the growth of amylolytic bacteria (Martin *et al*., 1984) and increased the population of cellulolytic bacteria (Dawson, 1990). The ability of yeast to stimulate the metabolism of lactate by *Megasphaera elsendi* and *Selenomonas ruminantium* contributes to stability of the rumen pH and efficiency of rumen function (Chaucheyras *et al*., 1995). Again, oxygen which is associated with plant particles and in saliva, enter into the rumen and is known to inhibit the growth and adhension of cellulolytic bacteria. Yeast is known to quickly utilize oxygen entrapped into open spaces and on the surface of ingested plant particles, thus creating better conditions for cellulolytic bacteria and decreasing the lag time in the cellulolysis process (Malcon & Kiesling, 1986). Oxygen uptake by yeasts is probably a major process involved in the improvement of a prime rumen function like cellulolysis.

From the above data, it is evident that *C. pubescens* had higher degradability than *P. maximum*. Factors such as type of plant, frequency of cutting, part of plant used and the age of plant used could have led to these differences the effect of the yeast notwithstanding. The age of the *P. maximum* could have influenced it’s degradability as older forages have more lignin, which interferes with rumen bacterial attachment and forage degradability.

Differences in the crude fiber contents of *P. maximum* and *C. pubescens* also accounted for better degradability of the later than the former at any given dose level of the bioactive yeast.

In conclusion, yeast *(S. Cerevisiae*) should be highly regarded as a potential manipulator of rumen function for effective rumen degradability of forages. It could therefore be used in ruminant diets to improve fibre breakdown particularly of poor quality feedstuffs.

References

Adams DC, Galyean ML, Kiesling HE, Wallace JD & Finker MD (1981). Influence of viable yeast culture, sodium bicarbonate and monensin on liquid dilution rate, rumen fermentation and feedlot performance of growing steers and digestibility in lambs. *J. Anim. Sci*. **53**:780-790.

Ademosun, AA (1973). Nutritive evaluation of Nigerian forages. The effect of stage of maturity on the nutritive value of *Panicum maximum* (Guinea grass). *Nig. Agric. J*. **10**:170-177.

Aka LO (2004). *Studies on the Rumen Degradability of Some Forage Plant Proteins and the Normal Rumen Outflow in sheep.* Masters Degree Dissertation. Department of Veterinary Physiology and Pharmacology, University of Nigeria Nsukka. Pp 125-140.

Association of Analytical Chemistry (AOAC) (1990). *Official methods of analysis, (15th edition*). Washington. D. C. Pp 1045-1052.

Agricultural Research Council (ARC) (1980). *The nutrient requirements of Ruminant Livestock*. Farnham Royal, Commonwealth Agricultural Bureaux. *Archives of Animal Nutrition,* Volume **47**(3):295 – 300.

Aregbede OM, Bamikole MA, Olanite JA, Jolaosho AO & Onifade OS (2002). Seasonal degradability of dry matter, organic matter and crude protein in some multipurpose tree species by West African dwarf goats. *Proc. Annul. Conf. Nig. Soc. Anim. Prod*. 191-194

Chaucheyras F, Fonty G, Bertin G & Gouet P (1995). Effects of Live *Saaccharomyces cerevisiae* cells on Zoospore Germination, Growth, and Cellulolytic Activity of the Rumen Aaerobic Fungus, *Neocallimastix frontalis* MCH3. *Current Microbiol.,* **31**:201-205.

Czerkawski JW (1986). *An introduction to Rumen Studies*. Oxford, Pergamon Press. Pp 122-132.

Dawson KA & Newman KE (1987). Effects of yeast cultures supplement on the growth and activities of rumen bacteria in continuous cultures. *J. Anim. Sci.* **65**l): 452.

Dawson KA (1990). Designing the yeast culture of tomorrow-mode of action of yeast culture for ruminants and non-ruminants. *In: Biotechnology in the Feed Industry. Vol. VI. (TP Lyon Editor)* Alltech Technical Publications, Nicholasville, Kentucky, USA, Pp 119-125.

Devendra C (1988). Sheep and Goat Production in Guyana. *Zeit. Tier. Zunch*. **92**:305-317.

Devendra C (1986) Feeding system and nutrition of goats and sheep in the tropics. *Zeit. Tier. Zunch.* **89**: 234-241.

Egbo IC (2008). *The In vitro method of studying the effect of calcium chloride on forage degradability of P.maximum and C. pubescens*. DVM Thesis, Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka.

Erasmus LJ, Botha MP, & Kistner A (1992). Effect of yeast culture supplement on production, rumen fermentation and duodenal nitrogen flow in dairy cows. *J. Dairy Sci.*, **75**:3056-3065.

Fickers P, Benetti PH, Wache Y, Marty A, Mauersberger S, Smit MS & Nicaud JM (2005). Hydrophobic substrate utilization by yeast and its potential applications. *Yeast Res.* **5**(6-7) 527-543.

Fiems LO, Cottyn BG, Dussert L & Vanacker JM (1993). Effect of viable yeast culture on digestibility and rumen fermentation in sheep fed different types of diets. *Repro. Nutr. Devel* **33**:43-49.

Henics, Z & Gombos, S (1991). Effect of yeast culture on milk production, feed digestibility and ruminal fermentation of dairy cattle. *In: Biotechnology in the Feed Industry. Vol. VII. (TP Lyon Editor*). Alltech Tecnical Publications, Nicholasville, Kentucky, USA. Pp 307-312.

Kumar U, Vijay K & Sadarshan S (1997). Effect of Yeast culture supplement on ruminal microbial populations and metabolism in buffalo calves fed high roughage diet. *J.Sci. Food Agric.* **73**:231-236.

Lufadeju EA & Olorunju SAS (1986). The ruminal degradation of some agro-industial by-products. *Nig. J. Anim. Prod.*, **6**(2):161-170.

Malcon KI & Kiesling HE (1986). Influence of live yeast culture and whole cottonseed on milk production and butterfat production of lactating dairy cows and rumen fermentation of steers. *J. Anim. Sci.* **63**(Suppl 1): 473.

Martin SA, Nisbet D & Dean G (1984). Influence of a commercial yeast supplement on the in vitro ruminal fermentation. *Nutr. Rep. Int.* **40**: 335-342.

Minson DJ (1990). *Forage in ruminant nutrition*. Academic Press , New York.

Morrison M, Murray RM & Boniface AN (1990). Nutrient metabolism and rumen microorganisms in sheep fed a poor quality tropical grass hay supplemented with sulfate. *J. Agric. Sci.* **115**:269-275.

Muhammad N, Maigandi SA, Hassan WA & Daneji AI (2008). Growth Performance and Economics of Sheep Production with varying Levels of Rice Milling waste. *Sokoto J. Vet. Sci.,* **7**(1):59-64.

Newbold CJ, Wallace RJ, Chen XB & McIntosh FM (1995). Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. *J. Anim. Sci*. **73**:1811-1818.

Newman KE & Spring P (1993). A comparison of two different strains of yeast in their ability to alter ruminal and ceacal fermentations. *J. Anim. Sci. 71(Suppl), 289.*

Orskov ER, Hovell FD & Mould F (1980). The use of nylon bag technique for evaluation of feedstuffs. *Trop. Anim. Prod.* **5**:195-213.

Santra A & Karim SA (2002). Rumen cannulation in sheep and goats: Fabrication of cannula and surgical procedure for its implantation. *Indian Journal Science* **72**:978-980.

Steel RDG & Torrie JH (1980). *Principles and procedures of statistics. A biometric approach*. McGraw Hill. Book Co, New York.

Smith OB (1988). Studies on the Feeding Value of Agro-Industrial By-products. Effects of forage supplementation on the utilization of cocoa pod based diets by ruminants. *J. Anim. Res,* ***8***(1):1-14.

Weidmeir RD, Arambel MJ & Walter JL (1987). Effects of yeast culture and *Aspergillus oryzae* fermentation extracts on ruminal characteristics and nutrient digestibility. *J. Dairy Sci,* **70**:2063-2068.

Williams PEV, Tait GAG, Innes GM & Newbold CJ (1991). Effects of inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. *J. Anim. Sci.* **69**: 3016-3026.