

## EFFECT OF *IN-VITRO* YEAST TREATMENT ON THE *IN-SACCO* DEGRADABILITY OF SOME TROPICAL CROP RESIDUES IN RUMEN FISTULATED WEST AFRICAN DWARF SHEEP

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### ABSTRACT

*The effect of in vitro bioactive yeast (Saccharomyces cerevisiae, strain 1026) treatment on the degradability of dry matter fraction of some crop residues using the in-sacco degradability technique in West African Dwarf sheep was studied. Sixteen (16) adult rumen fistulated WAD sheep of average body weight of  $16.84 \pm 3.43$  kg were used. Degradability of crop residues; rice straw, groundnut foliage, pigeon pea foliage and maize stover was studied. The results showed a significant ( $p < 0.05$ ) treatment effect. Time (length of yeast treatment) effect was positive for 2-6 day yeast treated groundnut foliage and pigeon pea foliage and for 8-10 day treatments for rice straw and maize stover. There was a negative correlation coefficient ( $r = -0.76$ ) between degradability and length of yeast treatment for 8 and 10 day treated groundnut and pigeon pea foliages. There was a positive correlation ( $r = 0.69$ ) between length of treatment and percentage degradation for all crop residues at 2, 4 and 6 day treatments. At 48 hours incubation, the degradability of pigeon pea ( $96.41 \pm 3.89$  %) and groundnut foliages ( $93.17 \pm 3.10$  %) were significantly ( $p < 0.05$ ) higher than rice straw ( $55.54 \pm 7.11$  %) and maize stover ( $61.08 \pm 6.42$  %) particularly at 4 and 6 days yeast treatment periods compared with the control. On the other hand, the rumen degradability of 8 and 10 day yeast treated rice straw ( $81.72 \pm 5.91$  % and  $94.20 \pm 2.81$  % respectively) and maize stover ( $89.93 \pm 4.16$  % and  $95.61 \pm 4.22$  % respectively) were significantly ( $p < 0.05$ ) higher than the control ( $49.06 \pm 4.11$  % for rice straw and  $43.18 \pm 4.78$  % for maize stover) at 72 hours but not 48 hours during which groundnut and pigeon pea foliages were optimally degraded following 4 and 6 day yeast treatments. It was evident from this study that length of in vitro yeast treatment, period of rumen incubation and type of crop residues had remarkable effects on rumen degradability of dry matter fraction. Thus, treating crop residues for appropriate periods, in vitro, with yeast cultures could provide opportunity for enhancing the fermentative potential and utilization efficiency of crop residues in ruminants generally but particularly in West African Dwarf sheep.*

**Keywords:** Yeasts, Degradability, Crop residues, Sheep

### INTRODUCTION

Antibiotics and growth promoting hormones and other chemical rumen manipulators have been used in ruminal diets to enhance productivity and performance (Nielsen and Thamsborg, 2005). Naturally occurring plant secondary

metabolites have shown potentials to improve rumen fermentation favorably and to increase feed efficiency, thus live weight of animals. A number of studies support the use of probiotics (lactic acid bacteria, such as lactobacilli and bifidobacteria, and yeast culture such as *Saccharomyces* spp, and other beneficial

bacteria to improve feed conversion and utilization (Fuller, 1989) as well as improve rumen fermentative activity (Williams *et al.*, 1991) and weight gain (Fallon and Harte, 1987), growth rate and feed conversion efficiency (Ramaswami *et al.*, 2005), microbial protein flow (El Hassan *et al.*, 1996) and dry matter intake (Putnam *et al.*, 1997).

Beneficial effects of yeast culture supplements have been associated with their abilities to alter rumen function favorably. Yeast culture may alter the patterns of volatile fatty acid (VFA) formation (Martins *et al.*, 1989), alter ruminal pH (Harrison *et al.*, 1988), increase the concentrations of anaerobic and cellulolytic bacteria (Wiedemeir *et al.*, 1987). Aseltine (1991) had earlier stated that though feeding probiotics has been more prevalent in non-ruminant livestock industries than in ruminant feeding, opportunities may exist for improving the health and growth of ruminants by their use. This therefore could apply in poor management conditions or to feedstuff such as crop residues that have high lignocellulose contents.

Generally, the utilization of crop residues for ruminant animals are limited by low nitrogen, low mineral and vitamin contents (Orskov, 1998; Oddoye *et al.*, 2005). In order to avert this limitation and further explore other ways of using yeast cultures to improve rumen degradability of some readily available crop residues, the effect of yeast cultures (commercial bioactive yeast) on rumen degradability of the dry matter of some crop residues was studied. The positive effects of yeast culture on the *in vitro* fermentation of diets containing variable portions of concentrates and crude fiber have been demonstrated (Carro *et al.*, 1992). On the other hand, adding yeast culture to the high concentrate diet increased dry matter (DM) and neutral detergent fiber (NDF) degradability, volatile fatty acid production, methane production and concentration of protozoa while output of ammonia was reduced (Carro *et al.*, 1992). These findings indicated that yeast culture influenced the rumen microbial population and had a diet dependent effect on the fermentation pattern. However, the effect of

extent of yeast treatment on the rumen degradability of the readily available and cheap crop residues in Nigeria has been scarcely studied. To further exploit the benefits of yeast culture, this study was aimed at evaluating the effects of period of *in vitro* yeast treatment on the rumen degradability of some of such crop residues.

## MATERIALS AND METHODS

**Animal:** Sixteen adult West African dwarf sheep purchased from Opi market in Nsukka, Enugu State, Nigeria, were weighed and quarantined for 14 days after which they were acclimatized for 21 days in the animal house of the Department of Veterinary Physiology / Pharmacology. They were fed twice (9.00 am and 3.30 pm) daily using fresh palm leaves only. Maize bran was occasionally supplemented. Water was provided *ad libitum* while salt was provided as a lick once (24 hour) a week. During the acclimatization period ectoparasites and endoparasites were routinely controlled with prescribed vaccination (Obidike *et al.*, 2009)

They were later divided into two groups, A and B after the 21day acclimatization period, with each group comprising of 8 sheep. All sheep in both groups were fitted with rumen fistula as described by Santra and Karim (2002).

**Yeast:** Commercial bioactive yeast, was a flocculent strain of *Saccharomyces cerevisiae* obtained from Nutfield, Surrey, UK and referred to as strain 1026. Each crop residue was treated by mixing 1 g of yeast with 1 kg of milled crop residue (Williams *et al.*, 1991) for 2, 4, 6, 8 and 10 days respectively. The *in-sacco* technique of rumen degradability (Ørskov *et al.* 1980) was used for *in-situ* studies. Procedures and protocols adopted for the rumen degradation were as described by Aregbede *et al.*(2002). Degradation constants of the dry matter (DM) were fitted into the formula of Ørskov and McDonald (1979) as given thus:  $P = a + b(1 - e^{-ct})$ , where, P is the disappearance at time t, a is the rapidly disappearing or immediate soluble fraction (i.e. zero time intercept), b is the slowly

degradable fraction of the feed at time  $t$  and  $c$  is the rate constant for the degradation of  $b$ .

**Statistics:** Treatment effect (period of *in vitro* yeast treatment) for each crop residue and forage effect within a treatment was statistically analyzed using the two way analysis of variance (ANOVA). Significant differences in mean degradability of a crop residue for different yeast treatment periods (in days) and between crop residues for a given treatment and for any given incubation period were tested using the least significant difference (LSD) method of mean comparison at 0.05 probability level (Steel and Torrier, 1980).

## RESULTS

The *in sacco* dry matter degradability of yeast-treated rice straw, groundnut foliage, pigeon pea foliage and maize stover treated differently with commercial bioactive yeast for various days are as given below. Table 1 showed the chemical composition of the crop residues before treatment with bioactive yeast. Rice straw and maize stover had a high dry matter (DM) content of 93.9 and 93.4 % respectively compared to groundnut and pigeon pea foliages with DM contents of 90.06 and 91.73 % respectively. The crude protein contents of groundnut foliage and cow pea foliage were 13.46 % and 14.74 % respectively. Each of them was higher than that of rice straw and maize stover.

The percentage disappearance of dry matter in rice straw residue between day 2 to 10 yeast treatments period are shown on Table 2. The table showed that there was significant ( $p < 0.05$ ) increase in percentage disappearance of DM for all the treatment periods compared with the control except for the 2 day treatment. However, 10 day yeast treatment gave the most significantly increased degradability at 48 hours ( $81.08 \pm 5.01$ ) and 72 hours ( $94.20 \pm 2.81$ ) incubation periods.

Table 2 showed the dry matter disappearance of maize stover treated with yeast culture for different incubation periods. Contrary to the observations made with rice straw, 6 and 8 day treatments consistently, at

all incubation periods, gave significantly ( $p < 0.05$ ) higher disappearance compared to the control and other treatments. At 72 hours post incubation, 10 day yeast treatment recorded a significant ( $p < 0.05$ ) decrease ( $45.61 \pm 4.22$ ) in DM disappearance compared to other treatments. The decrease however was not significantly different from the control.

It was observed that at 48 and 72 hours post incubation the disappearance of groundnut foliage was significantly ( $p < 0.05$ ) highest at 4 and 6 day treatments compared to 8 and 10 day treatments and control. The dry matter disappearance of 10 day yeast treated groundnut foliage was significantly ( $p < 0.05$ ) decreased compared to the control. There was no significant difference in the DM disappearance between 2, 8 day treatments and control (Table 2).

The dry matter disappearance in pigeon pea foliage was similar to that of groundnut foliage except for the quantitative values. In same manner, 4 and 6 day treatments recorded the highest percentage disappearances which were significantly ( $p < 0.05$ ) different from other treatments and the control. Also, there was significant reduction in dry matter disappearance in 10 day treated pigeon pea foliage compared to the control (Table 2).

## DISCUSSION

The chemical composition of the crop residues has shown that groundnut foliage and pigeon pea foliage have higher crude protein than rice straw and maize stover (Table 1). Conversely, the crude fiber contents of rice straw and maize stover were higher than that of groundnut and pigeon pea foliages. The higher crude protein content of groundnut and pigeon pea foliages suggests that they are of better quality than the rice straw and maize stover. Kitanyi and Owem (1993) have reported that residues from leguminous crops have a higher protein quality better than that of other crop residues and may be used as protein supplements to the poorer quality residue and to the mature pasture.

More so, the higher acid detergent fiber contents of rice straw and maize stover indicated their high lignocelluloses content

Table 1: Chemical composition (%DM) of untreated experimental crop residues

Crop residues	Percent proximate composition							
	Dry matter	Crude protein	ADF	NDF	Cellulose	Lignin	Ash	OM
Rice straw	93.9	5.81	60.83	70.82	44.21	5.69	8.16	85.8
Maize stover	93.46	5.93	58.44	82.63	50.62	9.68	17.14	76.32
G. Foliage	90.06	13.46	26.46	41.74	39.86	4.11	5.52	84.54
Pigeon pea foliage	91.73	14.74	27.57	39.86	41.02	4.38	5.58	86.15

ADF = acid detergent fiber, NDF = Neutral detergent fiber, OM = organic matter

Table 2: Dry matter disappearance of some crop residues treated for different days with bioactive yeast, in West African dwarf sheep

Period of yeast treatment (days)	% disappearance at time t (hours)			
	12	24	48	72
<b>Rice straw (RS) residue</b>				
RS(2days)	13.46±2.65	26.37±3.11	46.03±3.7 <sup>a,d</sup>	58.96±4.77 <sup>a</sup>
RS(4days)	18.94±4.87	30.68±4.09	55.54±4.21 <sup>a</sup>	62.15±3.40 <sup>a,b</sup>
RS(6days)	20.43±4.89	44.09±2.61	68.94±3.65 <sup>b</sup>	78.46±2.01 <sup>b,c</sup>
RS(8days)	20.96±1.66	39.66±3.98	71.17±2.87 <sup>b,c</sup>	81.72±5.91 <sup>c,d</sup>
RS(10days)	16.63±0.87	31.94±6.10	81.08±5.01 <sup>c</sup>	94.20±2.81 <sup>d</sup>
Control (untreated)	10.98±3.09	21.08±3.28	38.85±4.10 <sup>d</sup>	49.06±4.11 <sup>a</sup>
<b>Maize Stover (MS) residue</b>				
MS(2days)	10.86±1.04	18.48±2.65	53.09±3.66 <sup>a</sup>	59.93±3.12 <sup>a</sup>
MS(4days)	16.78±2.11	24.97±3.99	61.08±2.92 <sup>a</sup>	70.04±4.22 <sup>b</sup>
MS(6days)	20.07±3.71	41.63±2.61	71.96±5.02 <sup>a,b</sup>	83.56±3.89 <sup>c</sup>
MS(8days)	24.93±3.61	46.37±4.02	79.76±4.17 <sup>a,b</sup>	89.93±4.16 <sup>c</sup>
MS(10days)	16.78±2.99	30.09±3.91	39.67±3.75 <sup>c</sup>	45.61±4.22 <sup>d</sup>
Control (untreated)	15.74±2.89	26.01±3.66	36.08±4.26 <sup>c</sup>	43.18±4.78 <sup>d</sup>
<b>Groundnut (GF) foliage residue</b>				
GF(2days)	16.91±1.99	26.49±3.98	46.06±2.11 <sup>a</sup>	59.66±3.22 <sup>a</sup>
GF(4days)	20.81±2.10	56.41±2.01	93.17±4.1 <sup>c</sup>	96.74±.89 <sup>c</sup>
GF(6days)	28.39±3.02	45.01±0.93	86.03±4.91 <sup>c</sup>	89.96±1.93 <sup>c</sup>
GF(8days)	18.14±1.59	27.65±2.19	43.19±4.19 <sup>a</sup>	60.05±0.45 <sup>a</sup>
GF(10days)	10.84±3.02	16.88±1.97	24.18±2.95 <sup>b</sup>	33.96±0.38 <sup>b</sup>
Control (untreated)	16.73±2.36	26.73±2.99	49.78±2.88 <sup>a</sup>	57.39±1.04 <sup>a</sup>
<b>Pigeon pea (PP) foliage residue</b>				
PP(2days)	19.72±1.11	20.18±2.18	54.95±2.21	71.26±2.65 <sup>b</sup>
PP(4days)	22.18±0.91	47.83±0.92	96.41±1.44	—
PP(6days)	26.46±1.87	53.76±1.04	84.05±3.20	93.74±0.96 <sup>a</sup>
PP(8days)	20.93±0.83	30.46±2.31	44.75±2.89	66.47±1.56 <sup>b</sup>
PP(10days)	13.93±0.94	19.27±3.55	29.41±0.79	39.08±3.02 <sup>c</sup>
Control (untreated)	18.17±1.18	29.81±1.76	56.15±1.49	69.84±2.78 <sup>b</sup>

Means within column with same superscript are not significantly different ( $P < 0.05$ )

compared to the leguminous foliages. Results of this study have shown that yeast treatment improved the rumen degradability of the crop residues studied. Their pattern of rumen

degradability was however found to be dependent on extent of *in-vitro* yeast treatment; incubation time and type of crop residue (Figure 1). The result has indicated that the degradation

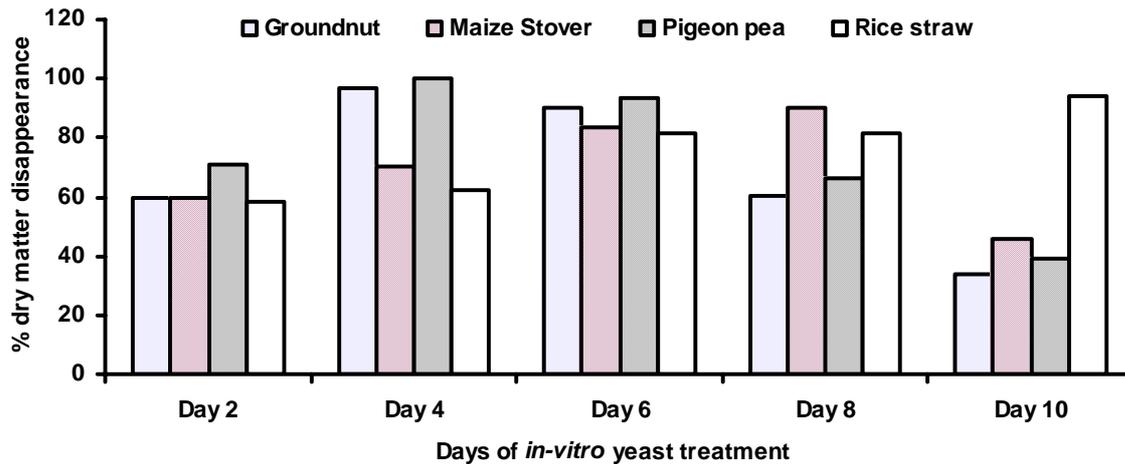


Figure 1: Disappearance of dry matter in different crop residues at 2 to 10 days of *in-vitro* yeast treatment

rate of yeast treated crop residues preceded more rapidly than the control (untreated crop residues). Classically, length of *in-vitro* yeast treatment affected the rate of degradation. Such differences in the degradability probably were due to variations in fiber contents of the yeast treated and yeast untreated crop residues. Though rice straw had a higher acid detergent fiber of 60.83 (% DM) compared to maize stover with acid detergent fiber of 38.94 (% DM), both required prolonged period of *in vitro* yeast treatment and more prolonged rumen incubation time for better *in-vivo* (intraruminal) fiber breakdown while groundnut and pigeon pea foliage with lesser acid detergent fiber (26.56 % and 27.5 % respectively) required lesser period of *in-vitro* yeast treatment and lesser rumen incubation time for high level degradation. The more the yeast was allowed to act (*in-vitro*) on the crop residues, the more the acid detergent fiber content of the crop residues was digested *in-vitro* prior to incubation.

It has been documented that the use of probiotics such as yeast in the pretreatment of feeds causes the expression of enzymes which exert *in-vitro* fibrolytic activity and in turn enhance rumen degradation of such treated feedstuffs (Dawson *et al.*, 1990). Feng *et al.* (1992 a,b) have reported that feed treatment methods and length of treatment enable adequate interaction between enzyme and substrate prior to feeding and are most likely to improve animal performance. This study lays much credence to these earlier assertions.

Enhanced degradation of enzyme treated forages have also been related to the decrease in rate of passage and increase in the retention time of forage particles in the rumen of cattle (Feng *et al.*, 1992b), thus with increase in retention time, exposure time of the digesta to rumen microbes would also be increased, paving way for effective microbial attack with consequent increase in degradability (Stewart, 1977). This probably played a role in the regulation of the degree of fiber breakdown of each crop residue especially as the yeast treatment could have affected their particle sizes prior to incubation hence the differences in their rumen retention time and DM disappearances. The *in-vitro* yeast treatment for the leguminous crop residues (GF and PP) probably led to high fiber breakdown *in-vitro* compared to the rice straw and maize stover residues hence variations in the pre-incubation particle sizes. We speculate that such activity probably increased the solubility of the immediate soluble fraction ('a') of the DM prior to incubation. As a result their percentage dry matter disappearance as incubation time increased was increased. Again the suspected decrease in particle sizes probably occasioned high outflow rate of the residues from the reticulorumen leading to decreased detectable feed fraction in the rumen and consequently an increase in the percentage disappearance.

The increase in the degradability of 4 and 6 day yeast treated groundnut foliage and pigeon pea foliage at 48 hours incubation

indicated that these leguminous foliages required lesser *in-vitro* yeast treatment periods than rice straw and maize stover to attain better degradability in the rumen. For all the crop residues, there was evidence, from this study that yeast treatment improved dry matter degradability with period of *in vitro* treatment identified as a key factor that regulated the rate of degradability. This is in agreement with the reports of other workers. Harrison *et al.* (1988) and Cheng *et al.* (1981) concluded that addition of yeast culture improved ruminal degradation of forages by increasing ruminal stability in forms of ruminal pH, lactate concentration, and acetate to propionate ratios. In this study, the ruminal pH was slightly elevated (not shown). Stabilization of the ruminal environment and slight elevation in ruminal pH have been described by Wiedmeir *et al.* (1987) and Dawson *et al.* (1990) as the reasons for increase in total anaerobic bacteria, and consequently increase in fiber degradability. In this study therefore, yeast treatment probably increased cellulolytic bacteria in the rumen and hence the improved degradability of crop residues under study. It is proposed that yeast remove some of the oxygen that occurs in ruminal fluid at various times during the daily feed cycle (Hillman *et al.*, 1985) and therefore prevent toxicity to the rumen anaerobes.

Pretreatment of these crop residues with *Saccharomyces cerevisiae* probably stabilized the rumen environment and thereby caused increase in population of the rumen cellulolytic bacteria. Wallace and Newbold (1993) indicated that the most reproducible effect of microbial feed additives (e.g. *S. cerevisiae*) is that they increase the viable count of anaerobic bacteria recovered from ruminal fluid. They demonstrated an increase of 50 to 100 %. Martin and Nisbet (1992) suggested that yeast apart from increasing cellulolytic bacterial numbers, also stimulate lactic acid utilizing bacteria by the decarboxylic acids present in the rumen, thus stabilizing the rumen for better fermentative activities. In another trial, Newbold *et al.* (1990) and Cheng *et al.* (1984) observed that addition of yeast culture to feedstuffs produced a reduction in the acetate: propionate ratio which provides further evidence to the

beneficial effects of yeast treatment particularly on fermentation stoichiometry in the rumen.

Against this backdrop, we are now focusing on; 1) the rumen cellulolytic bacteria biomass of West African Dwarf sheep under normal free range grazing system as well as under controlled feeding system using these crop residues when untreated and treated with live yeast cultures. 2) the effects of *in vitro* yeast treatment on the acetate: propionate ratio when these crop residues are fed. These would enable us provide clearer evidences on the influence of yeast treatment on rumen ecology and function in West African Dwarf sheep as well as provide explanation for the mechanism of action of yeast cultures in improving rumen degradability of these crop residues in particular and forages in general.

In conclusion, this study has demonstrated that pretreatment of ruminant diets (especially those of high roughage level) with bioactive yeast (*Saccharomyces cerevisiae*), for a maximum recommendable period of 8 days could provide opportunities for improved feed utilization and ruminant performance, particularly in tropical areas where crop residues are readily available but usually wasted, as it could improve rumen degradability of feed fractions particularly the dry matter fraction.

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