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ORIGINAL RESEARCH ARTICLE

Influence of processed neem fruit and yeast mixtures on performance and digestibility of West African dwarf sheep

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ABSTRACT
A feeding trial using sixteen (16) male West African Dwarf (WAD) sheep with an initial live weight of 14.41±2.54kg was conducted to determine the effect of processed neem fruit and yeast supplementation on their performance characteristics and nutrient digestibility. The rams were assigned to four dietary treatments of four rams per treatment consisting of control (T1), yeast alone at 5 g/d (T2), neem fruit alone at 5 g/d (T3) and yeast plus neem fruit at 5 g/d (T4). The dry matter (%) contents were 93.03, 93.04, 93.71 and 93.71 while the crude protein were 8.75%, 8.75%, 10.29% and 10.29% for diets 1, 2, 3 and 4 respectively. Feed at 5% body weight and water were offered in one ration a day. Daily feed intake and body weight changes were determined and feed efficiency calculated. On the 56th day, three rams per treatment were selected for metabolic study. Nutrients digestibility were calculated and computed while nitrogen retention study was determined. Results indicated that yeast and neem fruit supplementation generally improved intake and average daily weight gain in WAD sheep. Also, DM (56.71, 57.19 and 69.16%) and ME (7.63, 7.47 and 8.05MJ/kgDM) digestibility for diets 2, 3 and 4 differed significantly (P<0.05) from diet 1 (DM 49.74% and M.E 5.55MJ/kgDM). Also, CP digestibility for diet 4 (82.73%) differed significantly (P<0.05) from diets 1, 2 and 3 (72.79, 76.79 and 76.33%) respectively. The DM intake (g/day) across the treatments differed significantly (P<0.05) with the best obtained from animals fed diet 4 (898.32g/d) and the least from animals fed diet 1 (636.06g/d). The best nitrogen retention was obtained from animals fed diet 4 and diet 1 gave the least. Addition of yeast and processed neem fruit (T4) promoted average daily weight gain and performance was comparatively better than those on other diets.

Key words: Processed neem fruit, Yeast, Digestibility, Nitrogen Retention and West African Dwarf sheep

INTRODUCTION

Neem is an evergreen plant that is predominant in Nigeria. Neem possesses maximum useful non-wood products such as leaves, bark, flowers, fruits, seeds, gum, and oil and neem seed cake than any other tree specie. Biologically, neem has numerous bioactive ingredients with diverse applications. These bioactive ingredients are known to have antiallergenic, antidermatic, antifeedant, antiviral, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic, insecticidal, larvicidal, anti-implantation, nematocidal, spermatocidal and other biological activities. Biologically active principles isolated from different parts of the plant include: azadirachtin, meliacin, salanin, nimbin, valassin and many other derivatives of these principles (Schmuttere, 1990; Uko and Kamalu, 2001 and Lale, 2002). These compounds belong to natural products called triterpenoids. Bawa *et al*., (2006), Uko and Kamalu (2001) utilized neem seed cake as animal feed while Oforjindu (2006); Esonu *et al*. (2005, 2006) and Ogbuewu *et al*. (2010) investigated the use of neem leaf meal in poultry and Sokunbi and Egbunike (2000) fed the cake to rabbits. Despite the high crude protein , its incorporation in animal diets was discouraged due to their adverse effect on their performance because of the presence of bitter and toxic triterpenoids mainly nimbin, nimbidin, azadrachtin and salanin (Paul *et al*., 1996). Similarly, use of live microorganisms such as yeast as feed supplements for ruminants is not a new concept e. g. products containing yeast, particularly live yeast cells and yeast metabolites of *Sacchromyces cerevisiae* have been used to increase growth rate and milk production in domestic ruminants (Pinos-Rodriguez *et al*., 2008: Masek *et al*., 2008). Currently, the potential roles of specific microbial supplements have been better defined and there has been considerable interest in using preparations containing live microorganisms as feed supplements for ruminants (Dawson, 2002). Yeast products are widely utilized as feed additives for ruminant animals by enhancing dry matter (DM) intake and overall animal performance (Robinson, 2009). Yeast supplements stimulate the growth of beneficial microorganisms in the rumen like the numbers of total ruminal anaerobes (Dawson *et al*., 1990; Newbold *et al*., 1991; Girard, 1997 and Jouany, 2001) and cellulolytic bacteria (Harrison *et al*., 1988; Girard, 1997 and Jouany, 2001) have been increased with yeast cultures (YC). Blake (1993) and Girard (1997) reported that yeast improved the cellulolytic activities of rumen microorganisms in such a way that they increase their total numbers, improve fibre digestion, reduce lactate accumulation, reduce the concentration of oxygen in rumen fluid and improve utilization of starch supplied in the feeding ration. Furthermore, they inhibit the rate of volatile fatty acids production and, thus, increase the stability of rumen environment and improve the intensity of digestion. Also, yeast has directly stimulated rumen fungi, which may improve fibre digestion (Chaucheryas *et al*., 1995). The study was designed to determine the influence of processed neem fruit and yeast supplementation on the digestibility and nitrogen retention of WAD sheep

MATERIALS AND METHODS

Location of study

The experiment was carried out at the small ruminant unit, Teaching and Research Farm, University of Ibadan, Ibadan. The farm is located between latitude 7^0 2^0 north and longitude 3^0 50^0 east. The altitude is about 200 meters above the sea level. The location has a daily temperature range of 25 ºC to 32 ºC, a daily humidity range of about 75% - 85% depending on the season and total annual rainfall range of 2032-3048mm.

Experimental Animals

Sixteen WAD sheep aged between 6-7 months weighing 14.41±2.54kg were used for the experiment. The animals were obtained from the flock of animals kept in the sheep unit. Prior to commencement of the study, all the animals were given Ivomectin injection at 1mL/25kgBW to control endo- and ecto-parasite infestation. Oxytetracycline (L.A) was administered at 1mL/10kg BW through an intra-muscular route. The animals were housed in different pens equipped with feeding and watering facilities. Feed was given at 5% body weight while water was available free choice.

Experimental Diets:

Preparation of processed neem fruit

Neem fruit were acquired from Nguru market, Yobe State. Acquired fruits were sundried for some days after which they were soaked in water for 48 hours. Water in which the fruits were soaked was changed twice within 24 hours interval. After 48 hours, soaked fruits were washed and sundried for some days. Dried water-washed neem fruit were milled and ready to be used.

Data Collection

An adaptation period of 14 days was allowed before data was collected for 56 days during which time the animals were weighed weekly. The weight of an animal at the end of a particular week was used to calculate the weight of the feed that will be offered to that particular animal the following week. Before the digestibility experiment, the animals were kept on concrete floor pens and 5% of their body weight of feed of various treatments of formulated diet as seen in Table 1, T1 (Control – without neem and yeast), T2 (yeast 5g/day), T3 (neem 5g/day) and T4 (neem 5g/day+yeast 5g/day), in groups of four was given, for 109 days to evaluate their feed intakes and growth rates. After the trial, twelve rams were selected for the metabolism. All the rams were housed in specially constructed metabolic cages to facilitate separate collection of faeces and urine. The animals were fed at the same rate with three on each test diet. A preliminary period of seven days was allowed before faeces and urine collection which lasted for five days was started. A 10% aliquot sample of the faeces voided daily was taken and put in a freezer to prevent spoilage then samples milled and stored in airtight bottles until required for analysis, while urine was collected over a drop of concentrated H_2SO_4 solution in a plastic container.

Table 1: Gross composition of the experimental diets (%)

Ingredients	T1	T2	T3	T4
Dried cassava peels	54.00	54.00	49.00	49.00
Palm kernel cake	11.00	11.00	11.00	11.00
Brewers dried grains	10.00	10.00	10.00	10.00
Groundnut haulms	20.00	20.00	20.00	20.00
Processed neem fruit	-	-	5.00	5.00
Di-calcium phosphate	2.00	2.00	2.00	2.00
Urea	1.00	1.00	1.00	1.00
Premix	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00
Yeast(g)	-	5	-	5
Total	100	100	100	100

T1 = without yeast and processed neem fruit supplementation (control); T2 = with yeast supplementation; T3 = with processed neem fruit supplementation; T4 = with yeast and processed neem fruit supplementation; Yeast was added at 5g/day/animal

Samples not ready for immediate analysis were kept in a deep freezer at -5°C. Clean water was available ad libitum in plastic buckets placed at the corner of each metabolic cage. This solution prevented nitrogen losses by evaporation of ammonia from the urine. The daily volume was measured every morning and 10% aliquot taken for nitrogen determination.

Sample Analysis and statistical design

The milled samples (feed and faecal samples) were later analyzed for their proximate analysis using A.O.A.C (1990) method. Fibre fractions were analyzed using the procedure of Van Soest (1994). The effects of the supplementation on the animals were subjected to Analysis of Variance (ANOVA) in a completely randomized design. Differences between the treatment means were separated using Least Significant Difference. All data collected were subjected to a one way analysis of variance using SAS (1999) software package.

Statistical model

$Y_{ij} = u + T_j + E_{ij}$

Where, Yij = individual observation; u = general mean; Tj = effect of the jth treatment; Eij = experimental error

RESULTS AND DISCUSSIONS

The chemical composition (%) of the formulated diet is presented in Table 2. Treatments 3 and 4 tended to be higher numerically in the parameters measured while their value for crude protein obtained in this study was within the minimum protein requirement of 10 – 12 % recommended by (ARC, 1985) for ruminants but the values for Treatments 1 and 2 were low in all the parameters and CP requirement. Table 3 shows the effects of yeast and processed neem fruit supplementation on the performance of WAD sheep in terms of their feed intake, weight gain and feed conversion ratio. The average daily intake of the animals was significantly different ($P<0.05$) across the treatments ranging from 683.75g/day/animal in animals on diet 1 to 958.62g/day/animal in animals on diet 4. All the animals gained weight but the rate of weight gain across the treatments were not significantly different for animals on diets 1, 2 and 3 but significantly different from animals on diet 4 at

Table 2: Chemical Composition (%) of the experimental diets

Fractions (%)	T1	T2	T3	T4
Dry Matter	93.04	93.03	93.71	93.71
Crude Protein	8.75	8.75	10.29	10.29
Ether Extract	17.8	17.8	18.82	18.82
Ash	7.38	7.38	10.55	10.55
Neutral Detergent Fibre	62.56	62.56	69.82	69.82
Acid Detergent Fibre	48.27	48.27	59.45	59.45
Acid Detergent Lignin	15.75	15.75	20.13	20.13
Hemicellulose	14.26	13.64	10.37	10.37
Cellulose	32.55	32.55	39.32	39.32

T1 = without yeast and processed neem fruit supplementation (control); T2 = with yeast supplementation; T3 = with processed neem fruit supplementation; T4 = with yeast and processed neem fruit supplementation

Table 3: Effects of Yeast and Processed Neem fruit supplementation on performance characteristics of WAD sheep

Performance Characteristics	T1	T2	T3	T4	SEM
Initial Body Weight(kg)	14.68^{a}	19.50^{a}	20.25^{a}	19.88^{a}	1.04
Final Body Weight(kg)	18.73^{b}	23.25^{ab}	24.63^{ab}	26.38^{a}	1.11
Total Feed Intake(kg)	38.30^{c}	47.00^{b}	50.99^{ab}	53.68^{a}	1.62
Average Daily Intake(g)	683.75^{c}	839.32^{b}	910.58^{ab}	958.62^{a}	28.90
Total Weight Gain(kg)	4.05^{b}	3.63^{b}	4.25^{b}	6.50^{a}	0.42
Average Daily Gain(g)	72.32^{b}	70.00^{b}	80.00^{b}	120.00^{a}	6.29
Feed Conversion Ratio	10.56^{ab}	11.99^{a}	11.38^{a}	7.99^{b}	0.56

a,b,c: Means in the same row with different superscript are significantly different (p<0.05)
T1 = without yeast and processed neem fruit supplementation (control); T2 = with yeast supplementation; T3 = with processed neem fruit supplementation; T4 = with yeast and processed neem fruit supplementation

5% level of significance. The feed conversion ratio for animals on diet 4 was the least (7.99) and it was significantly different (P<0.05) from other treatments (10.56, 11.99 and 11.38 for diets 1, 2 and 3 respectively). There were significant differences (P<0.05) observed in the final body weight of the animals across all the treatments. It can be inferred from this study that yeast and processed neem fruit supplementation in diet 4 had complementary effects on the productivity of WAD sheep. Kudke *et al*., (1999) reported that there was significant increase in the growth rate of calves fed green fodder supplemented with neem and observed increase in the dry matter intake of the animals fed diets supplemented with neem. The researcher also stated that animals that were not supplemented with neem were more prone to parasitic infections which interfered with their productivity when compared to neem treated animals. Tipu *et al*., (2006) reported that neem works as growth promoter by killing parasites that hinder the growth of animal. Tipu *et al*., (2002) concluded and reported that addition of 0.3% ground neem fruit in broiler feed has tremendous efficiency in combating coccidiosis. The researchers also reported that neem fruit contains a compound known as margosate which is responsible for the breakdown of *Eimeria* life cycle. From the work of Tipu *et al*., (2006), it can be deduced that neem fruit has the potential to eliminate some micro-organisms that are not beneficial in the rumen of ruminant livestock and at the same time, make the rumen environment conducive for the growth and development of beneficial microbes. The removal or elimination of non beneficial microbes from the rumen and maintenance of the beneficial ones will increase feed digestibility which in turn will increase feed intake, microbial cell outflow from the rumen thereby increasing the live weight gain of the animal. Performance of meat producing animals is assessed by growth parameters such as average daily weight gain and feed conversion ratio. Yeast supplementation has been reported to have resulted in increases in average daily weight gain of beef and dairy cattle (Williams *et al*., 1991; Wohlt *et al*., 1991; Kung *et al*., 1997; Wohlt *et al*., 1998) and so also is neem (Tipu *et al*., 2006). Mir and Mir (1994) however reported negligible effects of yeast supplementation on growth parameters while Dawson (2000) reported an 8.7% increase in average daily weight gain of beef cattle when supplemented with yeast. The results of the effects of yeast and processed neem fruit supplementation on the digestibility of nutrients of WAD sheep are presented in Table 4. The digestibility co-efficient for dry matter, crude protein and ash were not significantly different (P>0.05) for diets 2 and 3 but differed significantly (P<0.05) from diet 1 and 4 with diet 4 having the highest values. Differences in ether extract and hemicellulose digestibility across the treatments were not significant (P>0.05). The digestibility percentages of acid detergent fibre, neutral detergent fibre, acid detergent lignin and cellulose for diets 2 and 3 were not significantly (P>0.05) different but differed significantly (P<0.05) from diet 1 and 4 with animals on diet 4 having the highest digestibility values. According to Table 4 which shows the effects of yeast and processed neem fruit on the digestibility of nutrients, it was observed that there was an increase in the nutrients digestibility co-efficient of diet 4 (processed neem fruit and yeast supplementation) when compared with the other treatments especially diet 1 (Control) which agrees with reports of Wohlt *et al*., 1991; Newbold *et al*., 1995; Wohlt *et al*., 1998 that yeast supplementation increases

Table 4: Effects of Yeast and Processed Neem fruit supplementation on the Digestibility of WAD sheep

Digestibility (%)	T1	T2	T3	T4	SEM
Dry Matter	49.74[b]	56.71[ab]	57.19[ab]	69.16[a]	2.79
Crude Protein	72.79[b]	76.79[ab]	76.33[ab]	82.73[a]	1.48
Ether Extract	73.36[a]	69.32[a]	71.27[a]	75.58[a]	1.23
Ash	53.02[b]	59.72[ab]	60.82[ab]	72.28[a]	2.67
Neutral Detergent Fibre	66.45[b]	69.42[b]	72.76[ab]	80.27[a]	1.88
Acid Detergent Fibre	64.93[b]	67.56[b]	73.83[ab]	81.17[a]	2.20
Acid Detergent Lignin	75.02[b]	73.70[b]	79.22[ab]	84.91[a]	1.58
Hemicellulose	71.51[a]	74.46[a]	65.72[a]	74.42[a]	1.58
Cellulose	59.93[b]	64.42[b]	70.92[ab]	79.21[a]	2.59
Organic Matter	39.94[b]	54.76[a]	55.67[a]	59.97[a]	2.49
Metabolisable Energy	5.55[b]	7.63[a]	7.47[a]	8.05[a]	0.32

a,b,c: Means in the same row with different superscript are significantly different (p<0.05)
T1 = without yeast and processed neem fruit supplementation (control); T2 = with yeast supplementation; T3 = with processed neem fruit supplementation; T4 = with yeast and processed neem fruit supplementation

the rate or extent of digestibility of feed, especially roughages or protein. This is however, at variance with the reports of negligible effects of yeast supplementation on nutrients digestibility by Putnam *et al*. (1997) and Doreau and Jouany (1998). Tipu *et al*., (2006) also reported that neem kills parasites that hinder the growth of animal, that is, neem has the potential to eliminate micro-organisms that hinder digestibility and nutrient utilization in the rumen. The dry matter in the diets was 93.04, 93.03, 93.71 and 93.71% for diets 1, 2, 3 and 4 respectively. Diets 1 and 2 were significantly different (P<0.05) from diet 3 and 4. Dry Matter Intake (DMI) across the treatments were significantly different (P<0.05) with diet 4 having the highest value of 898.32g/day and this agrees with the work done by Williams *et al*., (1991); Erasmus *et al*., (1992) that yeast supplementation in ruminant diets has led to increased dry matter intake (DMI) but does not agree with reports of no effects on DMI (Erasmus *et al*., 1989; Kung *et al*., 1997; Schingoether *et al*., 2004) or depression in DMI at high levels of supplementation (Besong *et al*., 1996). Although DMI is not an estimate of digestibility, it however, impacts digestibility directly and because it is thought that an increase in the rate of digestion may in turn increase the emptying rate of the rumen and therefore increase animal DMI (Wallace, 1996). DMI is an important factor in the utilization of feed by ruminant livestock and it is a critical determinant of energy intake and performance in small ruminants. Increased feed intake will lead to increased productive output of animals providing more nutrients to the animals. Dry matter and fibre digestibility increased with supplementation of either yeast or processed neem fruit but supplementation with both yeast and processed neem fruit gave the highest percentage value because of the combined effects exerted by yeast and neem in manipulating rumen environment for better degradability and this is in line with the report of increased digestibility of dry matter and fibre in ruminant in response to yeast supplementation (Lila *et al*., 2004) as against the report of no change in diet digestibility (Putnam *et al*., 1997).

Table 5: Feed, Dry Matter Intake and Faecal and Urinary Output of WAD sheep supplemented with processed neem fruit and yeast

Parameters	T1	T2	T3	T4	SEM
DM in Feed (%)	93.04[b]	93.03[b]	93.71[a]	93.71[a]	0.1
DM Intake (g)	636.06[d]	780.69[c]	853.31[b]	898.32[a]	29.97
Faecal output (g/d)	588.20[a]	745.13[a]	787.00[a]	647.40[a]	42.39
DM in Faeces (%)	58.33[a]	45.43[b]	46.47[b]	42.77[b]	1.99
Faecal DMO (g/d)	319.66[a]	337.95[a]	366.88[a]	276.81[a]	17.5
Urine (ml/d)	426.27[a]	939.93[a]	697.67[a]	355.13[a]	127.69

a,b,c,d: Means in the same row with different superscript are significantly different (p<0.05)
T1 = without yeast and processed neem fruit supplementation (control); T2 = with yeast supplementation; T3 = with processed neem fruit supplementation; T4 = with yeast and processed neem fruit supplementation

Table 6: Nitrogen Metabolism of WAD sheep fed diets supplemented with processed neem fruit and yeast

Parameters	T1	T2	T3	T4	SEM
Nitrogen intake (g/d)	8.91[c]	10.93[b]	14.02[a]	14.76[a]	0.74
Faecal nitrogen (g/d)	2.41[a]	2.54[a]	3.29[a]	2.55[a]	0.15
Urinary nitrogen(g/d)	1.03[a]	1.89[a]	1.95[a]	0.89[a]	0.28
Nitrogen retention(g/d)	5.47[c]	6.49[bc]	8.78[ab]	11.32[a]	0.79

a,b,c,: Means in the same row with different superscript are significantly different (p<0.05)
T1 = without yeast and processed neem fruit supplementation (control); T2 = with yeast supplementation; T3 = with processed neem fruit supplementation; T4 = with yeast and processed neem fruit supplementation

Yeast has also directly stimulated rumen fungi, which may improve fiber digestion (Chaucheryas *et al*., 1995). They increased the number of rumen protozoa and neutral detergent fibre digestion in steers fed straw-based diets (Plata *et al*., 1994). Table 5 shows the dry matter in feed, intake and dry matter output of WAD sheep supplemented with processed neem fruit and yeast. The dry matter in the feed was 93.04%, 93.03%, 93.71% and 93.71% for diets 1, 2, 3 and 4 respectively. Diets 1 and 2 were significantly different (P<0.05) from diets 3 and 4. The dry matter intake increased across the treatments in which there was significant differences (P<0.05) with diet 4 having the highest value 898.32g/day and diet 1, the lowest, 636.06g/day. Data on nitrogen metabolism of sheep given diets supplemented with processed neem fruit and yeast is presented on Table 6. The average nitrogen intake varied from 8.91 to 14.76g/day. Nitrogen retention values 5.47, 6.49, 8.78 and 11.32g/day were obtained for treatments 1, 2, 3, and 4 respectively. There were significant differences (P<0.05) across all the treatments with animals on diet 4 having the highest value (11.32g/day) for nitrogen retention and least value was obtained from animals on diet 1 (5.47g/day). Animals fed diet 4 (supplementation of neem fruit and yeast) had the lowest value (0.89) for urinary nitrogen output though not significantly (P>0.05) different from other treatments (1, 2, 3) which resulted in a higher retained nitrogen compared to animals on other treatments. Loss of nitrogen in urine originates from various sources such as rumen loss, the replacement of metabolic losses in the gut and the incorporation of dietary nitrogen into microbial nucleic acids. Urinary nitrogen is mainly composed of urea (65-90%) and the remaining consists of considerable amounts of allantoin, hippuric acid, creatinine, creatine and uric acid (Tamminga *et al*., 1995). However, reduction in urinary nitrogen excretion in animals on diet 4 may have positive impact to abate nitrogen pollution to the environment either as ammonia in the air or as nitrate in soil and ground water. Complementary effects of yeast and neem fruit in diet 4 improved the digestibility of crude protein, retention of nitrogen when compared with diets 2 and 3 and metabolisable energy intake of diet 4 (8.05) though not significantly different (P>0.05) from diets 3 (7.47) and 2 (7.63) goes in line with the report that yeast supplementation increases extent of digestibility of protein (Wohlt *et al*., 1998, Wohlt *et al*., 1991) and supplementation of processed neem fruit having the potential of improving nutritive value and reduces loss of energy through methane emission (Adewumi, 2009).

CONCLUSION AND RECOMMENDATIONS

In recent times, one of the major constraints of improved ruminant livestock production in Nigeria is the unavailability of feed, all year round. The feed scarcity is further aggravated during the dry season when available forages is dry and of very low quality. Results from this study showed that supplementation of the low quality feeds with neem fruit and yeast can maximally improve ruminant productivity. The supplementation improved the dry matter intake, efficient utilization of feed and optimized weight gain when yeast and neem fruit were supplemented in the same diet for ruminant livestock especially West African Dwarf sheep. It is recommended that neem fruit should be treated with hot water instead of cold water before milling to reduce some of the anti-nutrients contained as this will improve its utilization by livestock. Further investigation should be conducted to determine maximum level of inclusion of yeast and processed neem fruit in order to maximize ruminant productivity. More so, livestock farmers should be enlightened on the potential of processed neem fruit and yeast as supplements during dry season so as to maintain ruminant productivity all year round.

Conflict of Interest

The authors wish to declare that there are no conflicts of interest as regards this publication.

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