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Response of albino rats fed varying levels of enzyme supplemented tigernut meal based diets.

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Abstract

A 21-day feeding trial to assess the effect of replacing 0,10,20,30 and 40% maize with enzyme supplemented tigernut meal on the performance, nutrient digestibility, haematological traits, serum chemistry and carcass yield of one hundred and eight albino rats of the wistar strain was conducted in a complete randomised design. Nine experimental diets (1, 2, 3, 4, 5, 6, 7, 8 and 9) were formulated in all. Diet 1 contained 40% maize + 0% tigernut meal without enzyme supplementation. Diets 2,3,4,5 and 6,7,8,9 were constituted by replacing maize at 10,20,30 and 40% levels with tigernut meal supplemented with 0.05% roxzymeG® and enzyme extract respectively. The results revealed that of all the parameters considered, only the gut characteristics were not significantly (P>0.05) influenced by the dietary treatments. Diets 5 and 9 (40% enzyme supplemented tigernut meal) proved to be the best by having the best performance and nutrient digestibility coefficients. It was then concluded that 40% inclusion level of enzyme supplemented tigernut meal as a replacement for maize proved to be better in rats than diets that contained 0, 10, 20 and 30% levels of enzyme supplemented tigernut meal based diets.

Key works: Albino rats, enzyme supplement tigernut meal, performance

Introduction

Inspite of the nutritional significance of crude fibre in the diets of non-ruminants and man, research findings (Anyaechie and Madubuike, 2004) revealed that dietary fibres are highly indigestible in the gastrointestinal tract of monogastric animals and therefore have little nutritional value. High fibre diets have also been found to have a longer transit time in the intestine of animals as well as a reduction in the digestibility of other nutrients (Farrel and Johnson, 1983).

Recently, breakthroughs in biotechnology and animal nutrition have revealed that exogenous enzyme supplementation renders the non-starch polysaccharides (NSP) which are resistant to the digestive enzyme of monogastric animals digestible. These non-starch polysaccharides (NSP) form the bulk of the carbohydrate portion of most feed ingredients of plant origin and account for 70-95% of the cell wall (Maust *et al* 1972). However, the inability of monogastirc animals to digest water insoluble fraction of non-starch polysaccharides (NSP) and mixed linked beta-D- glucans and pentosans as well as the negative correlation between the metabolisable energy coefficient and the non-starch polysaccharides (NSP) contents of various feed ingredients, makes the use of *Trichoderma viride* (roxazyme) in hydrolysing the polysaccharide structure of the cell wall an imperative (Broz *et al* 1993). Against this backdrop, this study was designed to investigate the response of growing albino rats on the dietary levels of enzyme supplemented tigernut meal based diets.

Materials and Methods

Sources of enzymes

RoxazymeG®, a commercially prepared enzyme was purchased from Roche Pharmaceutical Plc, Lagos.

Enzyme extract preparation

The white sorghum grains used for the preparation of the enzyme extract was purchased from Kuto market in Abeokuta, Ogun State of Nigeria. The grains were cleaned properly to remove dirt and sand by sieving, while lighter particles were removed by winnowing. The cleaned grains were then soaked in excess water for 48 hours at room temperature until maximum swelling of the grains was achieved. The water was changed twice to remove carbondioxide and soluble toxins as well as to reduce microbial growth. The water was then drained and grains were rinsed with clean water. Thereafter, the grains were spread on a clean cloth or mesh material to allow circulation of air and they were covered with another moist cloth until sprouts were 0.5 – 1.0cm in length. The germinated sorghum grains (malted sorghum) were washed with clean running water and oven dried at 70°C until 8% moisture content was obtained. The grains were later devegetated by removing the rootlets and milled by using a power driven milling machine sieved with 0.25mm wire mesh. The powdery form of the malted sorghum obtained at this point contains active enzymes which can degrade high fibre components of feedstuffs.

Experimental diets

A total of nine treatment diets (1,2,3,4,5,6,7,8 and 9) were formulated. Diet 1 contained 40% maize as the major source of energy with no enzyme supplementation, whereas diets 2,3,4 and 5 contained 10,20,30 and 40% replacement levels of maize with tiger nut meal supplemented with 0.05% roxazymeG®. Similarly, diets 6,7,8 and 9 were constituted by replacing maize with 10,20,30 and 40% tigernut meal and 0.05% enzyme extract supplementation. All the diets were formulated to represent the normal starter diet for broilers (Table1).

Experimental animals and design

A total of one hundred and eight weanling albino rats of the wistar strain with average initial weight of 52.0 - 52.5g were used in a 21 – day feeding trial. The rats were allotted separately to each treatment diet in a complete randomised design (CRD). Each treatment diet had three replicates with four rats each. The rats were housed in a metabolic cage and the rats had access to clean water and treatment diets *ad libitum*.

Digestibility study

A five day total urine and faecal collections was carried out on the last week of the experimental period. During the period, a specific quantity of the treatment diets were supplied to the rats and daily feed intake was recorded. Faeces and urine were collected daily, while the faecal collections were weighed and oven dried at 100°C for 24 hours. The total faecal collections for each treatment was pooled, mixed thoroughly and milled, while the urine collected from each rat per treatment were also mixed together with the addition of two drops of concentrated tetraoxosulphate (vi) acid to prevent drying off of the urine. Samples of the test diets, faeces and urine were analysed for proximate compositions and nitrogen contents according to AOAC (2000).

Haematological study

Two rats per treatment were randomly selected, weighed, decapitated and bled. About 5ml of blood samples were collected into EDTA (Ethylene diamine tetra acetic acid salt) labelled bottles for haematological parameters, while a second set of blood samples was collected in heparinised tubes for serum chemistry evaluation. Packed cell volume (PCV), haemoglobin (HB) concentration, red blood cell (RBC) count, white blood cell (WBC) count, total protein, albumin, creatinine and urea were determined as described by Brij *et al* (1990).

Carcass evaluation

At the end of the feeding trial, each rat was weighed, decapitated, bled and eviscerated to obtain empty carcass weight. The weights of the liver, lungs, kidneys, spleen and heart were taken and related to their body weights. While, the length of the gastrointestinal tract (GIT) was measured using a meter rule and later weighed.

Statistical analysis

Data generated on performance, nutrient digestibility, haematological, serum biochemical traits and carcass yield were subjected to a one-way analysis of variance as descried for complete randomised design (CRD) and significant treatment means were separated using the Duncan's multiple range test (Steel and Torrie, 1990) using the SAS (1999) package.

Results and Discussion

Performance data (Table 2) of rats fed the dietary treatments revealed that final liveweight, feed intake, daily weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER) and protein intake of rats fed 40% tigernut meal supplemented with roxazymeG® and enzyme extract were significantly ($P<0.05$) higher than those obtained in rats fed the control diet. Infact, all the values obtained in these parameters were

Table 1: Percentage composition of experimental diets

	Tigernut RoxazymeG® inclusion(%)					Tigernut Enzyme extract inclusion (%)			
	0	10	20	30	40	10	20	30	40
					Diets				
Ingredients	1	2	3	4	5	6	7	8	9
Maize	40.00	30.00	20.00	10.00	0.00	30.00	20.00	10.00	0.00
Tigernut meal	0.00	10.00	20.00	30.00	40.00	10.00	20.00	30.00	40.00
Maize offal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soyabean meal	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Fishmeal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Rice husk	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Bone meal	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Oyster Shell	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Roxazyme G®	0.00	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00
Enzyme extract	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated M.E.)Kcal/g)	2.75	2.66	2.57	2.47	2.38	2.66	2.57	2.47	2.38
Determined analysis									
Crude protein	22.44	22.35	22.26	22.18	22.10	22.35	22.26	22.18	22.10
Crude fibre	4.20	4.83	5.46	6.09	6.72	4.83	5.46	6.09	6.72
Ether extract	3.73	5.07	6.40	7.75	9.09	5.07	6.40	7.75	9.09
Ash	4.53	4.84	5.16	5.47	5.78	4.84	5.16	5.47	5.78
NFE	54.40	51.69	47.98	46.27	43.56	51.69	47.98	46.27	43.56
Calcium	1.03	1.05	1.06	1.07	1.09	1.05	1.06	1.07	1.09
Phosphorous	0.68	071	075	078	081	0.71	0.75	0.78	0.81

Table 2: Performance characteristics of rats fed dietary treatments

	Tigernut – Roxazyme based						Tigernut – Enzyme extract based				
	Diets										
	1	2	3	4	5		6	7	8	9	
	Inclusion levels (%)						Inclusion level (%)				
Parameters	0	10	20	30	40	S.E.	10	20	30	40	**S.E**
Initial weight (g)	80.50	80.25	80.35	80.50	80.45	-	80.50	80.45	80.25	80.45	-
Final weight (g)	125.70^{c}	145.75^{d}	158.95^{c}	176.50^{b}	210.95^{a}	2.19	158.40^{c}	160.3^{b}	163.05^{b}	182^{a}	1.86
Feed intake (g)	14.56^{c}	14.96^{c}	15.0^{b}	15.06^{b}	15.77^{a}	0.28	15.03^{b}	15.22^{a}	15.53^{a}	15.53^{a}	0.25
Weight gain(g)	2.15^{c}	3.12^{b}	3.00^{b}	3.15^{b}	3.99^{a}	0.47	3.21^{b}	3.29^{b}	3.38^{a}	3.47^{a}	0.51
FCR	6.77^{a}	5.26^{b}	4.81^{c}	4.78^{c}	3.74^{d}	0.89	4.72^{b}	4.66^{c}	4.61^{c}	4.39^{d}	0.08
PER (%)	0.66^{e}	0.76^{d}	0.83^{c}	0.93^{b}	1.21^{a}	0.13	0.96^{b}	0.97^{b}	0.97^{b}	1.02^{a}	0.16
Protein intake (%)	3.27^{c}	3.30^{c}	3.34^{b}	3.36^{b}	3.51^{a}	0.06	3.36^{b}	3.42^{a}	3.43^{a}	3.47^{a}	0.06

abcde: Means in the same row with varying superscripts differ significantly ($P<0.05$)

Table 3: Nutrient digestibility of rats fed dietary treatments

	Tigernut-Roxazyme based						Tigernut-Enzyme extract based				
	Diets										
	1	2	3	4	5		6	7	8	9	
	Inclusion levels						Inclusion levels				
Parameters(%)	0	10	20	30	40	S.E	10	20	30	40	S.E
Dry matter	60.17^{c}	64.04^{b}	64.85^{a}	65.65^{a}	66.11^{a}	0.01	64.96^{b}	65.75^{a}	66.30^{a}	68.30^{a}	0.01
Crude protein	50.35^{d}	50.69^{d}	54.77^{c}	63.7^{b}	70.40^{a}	1.04	48.61^{d}	49.50^{c}	54.52^{b}	69.83^{a}	0.82
Ether extract	35.00^{e}	48.30^{d}	61.40^{c}	74.80^{b}	88.70^{a}	1.42	48.50^{c}	54.60^{b}	75.30^{a}	78.40^{a}	1.11
Crude fibre	50.40^{e}	55.20^{d}	57.45^{c}	60.25^{b}	63.46^{a}	0.99	51.45^{c}	55.23^{b}	59.63^{a}	59.63^{a}	0.81
Ash	23.40^{e}	37.80^{d}	43.30^{c}	45.30^{b}	49.60^{a}	0.05	36.70^{d}	38.20^{c}	39.80^{b}	42.60^{a}	0.38

abcde: Means in the same row with varying superscripts differ significantly ($P<0.05$)

observed to increase with increase in the inclusion levels of tigernut meal from 10 to 40% supplemented with roxazymeG® and enzyme extract . However, the improvement in feed and protein utilization by the experimental animals may be due to the enzyme supplementation of tigernut meal based diets which perhaps culminated in the higher weight gained by the rats than those on the control diets (40% tigernut meal + 0% enzyme). This support the assertion that feed conversion efficiency is inversely related to increased intake level because higher intake allows for increased fat deposition (Hammond *et al* 1971). The significant improvement in the performance of rats fed the tigernut based diets supplemented with roxazymeG® and enzyme extract compared to those fed the control diet could be due to the breakdown of the high fibre content of tigernut meal by both enzymes thereby making nutrients available at the tissue level (Onifade and Babatunde, 1997). This lend support from the findings of Simmons and Versteegh, (1993) who observed a significant improvement in growth rate and feed utilization of broilers fed pelleted barley supplemented with 40% *Trichoderma viride* derived enzyme complex (glucanase and xylanase activities).

Nutrient digestibility results as reflected in Table 3 indicated significant ($P<0.05$) increase in dry matter, crude protein, ether extract, crude fibre and ash digestibility coefficients with increase in the levels of enzyme supplemented tigernut meal inclusions. It was observed that increasing tigernut meal levels up to 40% with enzyme supplementation improved feed intake. feed utilization and nutrient digestibility. This suggests that the supplementation of tigernut meal with roxazymeG® and enzyme extract enhanced the degradation of the endosperm cell wall and the non-starch polysaccharide (NSP) constituent of the feed which eventually resulted to the rapid digestion of starch, protein and other nutrients in the small intestine of the rats (Ray *et* al 1982; Petterson and Aman, 1985).

The haematology and serum chemistry of rats fed the dietary treatments are shown in Table 4. Rats on 40% tigernut meal supplemented with roxazymeG® and enzyme extract produced the best ($P<0.05$) packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) when compared with rats fed the control, 10, 20 and 30% tigernut meal supplemented with enzymes. The extent to which these parameters were affected by the dietary treatments vary in accordance with the level of enzyme supplemented tigernut meal inclusion. This could be as a result of the fact that haematology reflects the physiological responsiveness of animals to both environment and nutrition (Eggum, 1980). The poor haematological indices of rats placed on the control diet (1) may be ascribed to the inability of the rats to obtain enough nutrients from the poorly digested diets. The serum constituents followed the same pattern as the haematological indices. The improvement in both the haematological and serum biochemical indices of rats could be adduced to the improvement in the digestion of the component nutrients in tigernut meal by the rats due largely to enzyme (Roxazyme G® and enzyme extract) supplementation. Furthermore, the reduction in the creatinine and urea values of rats as the level

Table 4: Haematological indices and serum chemistry of rats fed dietary treatments

	Tigernut-Roxazyme based						Tigernut-Enzyme extract based				
	Diets										
	1	2	3	4	5		6	7	8	9	
	Inclusion levels						Inclusion levels				
Parameters (%)	0	10	20	30	40	S.E	10	20	30	40	S.E
PCV (%)	26.7^{c}	27.6^{c}	37.8^{b}	39.0^{a}	40.9^{a}	0.70	37.6^{c}	41.5^{b}	42.4^{b}	44.5^{a}	1.20
Hb(g/dl)	8.7^{d}	9.1^{c}	13.1^{b}	13.3^{b}	16.7^{a}	0.20	12.3^{b}	13.8^{a}	14.0^{a}	14.7^{a}	0.20
RBC (x10^{12}/l)	3.00^{d}	3.10^{d}	4.40^{c}	4.50^{b}	5.70^{a}	0.11	4.20^{b}	4.60^{a}	4.80^{a}	5.00^{a}	0.11
WBC (X 10/^{12}/l)	3.60^{e}	4.40^{d}	4.80^{c}	5.20^{b}	6.20^{a}	1.10	5.00^{c}	5.60^{b}	5.80^{b}	6.40^{a}	0.11
Total Protein (mg/dl)	108^{d}	112^{c}	117^{c}	121^{b}	130^{a}	1.20	59^{c}	62^{d}	70^{c}	76^{b}	0.05
Creatinine (mg/dl)	2.90^{a}	2.70^{b}	2.60^{c}	2.50^{d}	2.40^{e}	0.06	2.60^{b}	2.30^{c}	2.20^{d}	2.00^{e}	0.06
Urea (mg.dl)	6.00^{a}	4.80^{b}	4.70^{b}	3.20^{c}	3.10^{c}	0.01	5.00^{b}	4.90^{b}	4.40^{c}	3.40^{d}	0.10

abcde: Means in the same row with varying superscripts differ significantly ($P<0.05$)

Table 5: Carcass and gut characteristics of rats fed dietary treatments

	Tigernut-Roxazyme based						Tigernut-Enzyme extract based				
	Diet										
	1	2	3	4	5		6	7	8	9	
	Inclusion levels						Inclusion levels				
Parameters (%)	0	10	20	30	40	S.E	10	20	30	40	S.E
Eviscerated weight	72.67^{e}	109.90^{d}	115.15^{c}	124.45^{b}	151.99^{a}	2.93	109.80^{d}	123.05^{c}	129.80^{b}	135.05^{a}	0.68
Dressing	71.90^{c}	80.90^{b}	81.90^{b}	81.43^{b}	87.05^{a}	0.78	80.77^{b}	81.86^{b}	83.65^{a}	83.88^{a}	0.84
Liver weight	4.64	4.20	4.10	4.25	4.30	0.27	4.80	4.65	4.45	4.50	0.37
Lungs	1.50	1.50	1.45	1.44	1.47	0.11	1.34	1.30	1.32	1.31	0.11
Spleen	0.50	0.51	0.55	0.53	0.52	0.10	0.46	0.44	0.43	0.43	0.05
Heart	0.50	0.50	0.50	0.51	0.52	0.05	0.65	0.60	0.64	0.63	0.06
Kidneys	1.05	0.95	0.98	0.85	0.95	0.08	0.90	0.97	0.93	0.90	0.07

abcde: Means in the same row with varying superscripts differ significantly ($P<0.05$)

of tigernut meal based diets supplemented with the enzymes indicate that there was no observable muscular wastage due to inadequate protein (Eggum, 1970). However, the value obtained for all the parameters compared favourably with the haematological standard values of rats (Mitruka and Rawnsley, 1977).

The carcass and gut characteristics as depicted in Table 5 revealed significant ($P<0.05$) increase in dressing percentage and eviscerated weights of rats as the level of enzyme supplemented tigernut meal increases. The relative weights of the liver, lungs, heart and kidneys were not significantly ($P>0.05$) affected by the treatment diets when compared with that of the control.

Conclusion

The overall result of this study indicate that enzyme supplementation in 40% tigernut meal as a replacement for maize enhanced the performance and nutrient digestibility of rats relative to diets containing 40% maize and 0.0% enzyme supplementation. This suggests that enzyme supplemented tigernut meal resulted to diets with higher digestible energy and crude proteins and of great utility.

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