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RESPONSE OF BROILER CHICKENS TO SAND BOX SEED MEAL BASED - DIETS SUPPLEMENTED WITH OR WITHOUT ENZYME

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ABSTRACT

This study examined the growth performance, carcass and blood characteristics of broiler chickens fed sand box seed meal - based diets supplemented with or without enzyme (Allzyme®Vegpro5X). A total of 240 unsexed day - old broiler chicks were randomly allotted to three dietary treatments in a completely randomized design experiment. There were four replicates of 20 birds each. The three experimental diets were formulated such that the control diet (T_1) had neither sand box seed meal nor enzyme, while soybean meal was replaced with sand box seed meal (100%) without enzyme (T_2) and soybean bean meal replaced with sand box seed meal (SBSM) supplemented with enzyme (1g/kg of feed) (T_3) for both starter and finisher diets. Performance parameters were monitored during the feeding trial. Results at the starter phase showed significant ($p<0.05$) differences in feed intake. At the finisher phase feed intake values showed non-significant ($p>0.05$) differences. The final live weight (FLW), daily weight gain (DWG), feed conversion ratio (FCR) and protein efficiency ratio (PER) all tended to significantly ($p<0.05$) increase in birds fed control diets. Nutrient retention values for all the parameters were significantly ($p<0.05$) lower with birds on SBSM - based diet without enzyme, while those of the control and SBSM - based diets with enzyme were numerically similar and significantly ($p<0.05$) higher than that of birds on SBSM - based diets without enzyme. The cost of feed per Kg live weight gain (₦) was lower for birds on SBSM - based diet supplemented with enzyme. Result of haematological parameters were significantly different ($p<0.05$) across dietary treatments for white blood cell counts (WBCs), lymphocytes, red blood cell counts (RBCs), haemoglobin concentration, haematocrit, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). Furthermore, all serum biochemical indices except total protein and bicarbonates showed significant ($p<0.05$) differences between dietary treatments. This study concluded that it is cheaper to produce one kilogram of broiler birds by replacing soybean meal with sand box seed meal supplemented with Allzyme®Vegpro5X without any adverse effects on the growth performance, carcass quality, nutrient retention and blood characteristics of broiler chickens.

KEYWORDS: Blood, chicken, enzyme, growth, sand box

INTRODUCTION

The major constraint to the expansion of poultry industry in Nigeria has been assessing feeds at affordable cost to meet the basic nutrient requirements of birds (Igene and Ekundayo, 2010). The general shortage of energy and protein feedstuffs, appear to be more severe for non-ruminants that depend to a greater extent on concentrate feeds. In view of this constraint and the present cost of feeding conventional energy and protein feedstuffs to poultry; increased utilization of

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non-conventional feedstuffs is inevitable (Adesehinwa *et al*., 2008; Adesehinwa *et al*., 2010). There exists in Nigeria, some non-conventional feedstuffs that can be used as total or partial replacement for the conventional ingredients in poultry feeds (Nsa *et al*., 2016). One of such is sand box (*Hura crepitans*) seed meal. Sand box tree is a shady tree with a thorny trunk, commonly found on road sides, in towns and villages within the Southern parts of Nigeria (Nsa *et al*., 2016). The woody segment fruit is like garden egg in shape of about 18-20 mm in diameters and of dry matter of about 91-95 %, which is quite comparable to those of the conventional feedstuffs (Keay, 2010).

It has crude protein (27 – 32 %), high in oil (38.95 - 51.25 %) and contains comparable amino acid contents with the other conventional seeds like soya bean and it is better in lysine, methionine, cystine, threonine and histidine contents (Yaakugh *et al.*, 2001). However, in most cases their utilization is limited as a result of their fibrous nature and high content of non-starch polysaccharides (NSPs) content (Nsa *et al.*, 2018). These NSPs are such that the enzymes present in the gastro-intestinal tract of the birds cannot fully digest or absorb (Bedford and Classen, 1992). Therefore, exogenous enzymes added to the feed or used during feedstuff processing have the ability to improve their efficiency by solubilizing the cell wall of fibrous feeds and reducing the viscosity of the intestinal ingesta (Bedford and Classen, 1992; Edache *et al*., 2018).

One of such exogenous enzymes that could be added to improve the nutritive value of plant -based feeds is Allzyme (R) Vegpro 5×. It is a combination of naturally occurring enzymes such as protease, cellulase, pentosanase (xylanase), galactosidase and amylase (Campbell and Bedford, 1992).

This study therefore investigated the effect of total replacement of soya bean meal with sand box seed meal in diets supplemented with or without Allzyme (R) Vegpro 5× on the growth performance, cost benefit, haematological indices and serum metabolites of broiler chickens.

MATERIALS AND METHODS

Study location

The study was conducted at the Poultry Unit of Teaching and Research Farm of the University of Calabar. Calabar is located within the tropical rain forest zone of Nigeria; it lies between latitude $4^0 5^{/}$ N and $15^0 39^{/}$ N of the equator and longitude $8^0 17^{/}$ E and $10^0 45^{/}$ E of the Greenwich meridian. It has an average relative humidity of 88 % with an average yearly rainfall of 3,076 mm (121.1 inches) within average yearly raining days of 173. The mean daily sunshine hours per day in Calabar is 3.8 hours while its average (high) temperature is 30.01^0 C (86.2^0 F) and average (low temperature of 22.5^0 C (72.5^0 F), with an elevation of 32 meters (105 ft) above sea level (Nigerian Meteorological Agency, NMA, 2020).

Collection and processing of sand box seed meal

The mature sand box seeds were harvested from sand box trees within Calabar metropolis. The raw seeds were harvested after breaking the pods with sticks; the seeds were sun- dried to a constant moisture level of 10 % before being milled in a meadow model 35 hammer mill and sieved through a mesh of 5 mm. Oil was extracted from the seed through mechanical means as described by Nsa *et al*. (2019). Some of the sand box seed meal was mixed with Allzyme®Vegpro5× at 1g/kg of feed. The samples were then stacked in sack bags for further assays and incorporation into the diets.

Experimental diets and animals

Three basal broiler starter and finisher mashes (Table 1) were formulated to provide the required nutrients of starter and finisher diets. Treatment 1 served as the control (soya bean-based diet without sand box seed meal and enzyme), while in treatments 2 and 3, soya bean meal was completely replaced (100 %) with sand box seed meal without enzyme and sand box meal supplemented with enzyme (Allzyme$^{(R)}$ VEGPRO 5×) at a recommended level 1g/kg feed, respectively (Aguihe *et al*., 2018). Allzyme®vegpro5× is a combination of naturally occurring enzymes like protease, cellulase, pentosanase (xylase), galactosidase and amylase.

A total of 240 unsexed day-old broiler chicks of “Fidan” strain were randomly allotted to 3 dietary treatments with four replicates of 20 birds each on weight basis, in a completely randomized design (CRD). The birds were properly vaccinated against common poultry diseases and housed in a deep litter pen with wood shavings as litter material. The birds were fed the experimental starter diets for 28 days, after which they were re-randomized on weight basis into the same treatments and fed finisher diets, which lasted for another 28 days. Feed and water were given *ad libitum* throughout the experimental period. The study was conducted following the guidelines of the Research Policy of the University of Calabar on animal care, welfare and ethics.

Table 1: Gross composition of experimental broiler chicken diets (%)

	Starter diets			Finisher diets		
Ingredient	T_1	T_2	T_3	T_1	T_2	T_3
Yellow maize	54.25	54.25	54.25	57.00	57.00	57.00
Soybean meal	32.25	-	-	28.50	-	-
Sand box seed meal (SBSM)	-	32.25	-	-	28.50	-
Sand box seed meal + Enzyme	-	-	32.25	-	-	28.50
Wheat offal	4.50	4.50	4.50	6.00	6.00	6.00
Palm kernel cake	2.75	2.75	2.75	3.00	3.00	3.00
Fish meal	2.50	2.50	2.50	2.00	2.00	2.00
DCP	2.50	2.50	2.50	2.50	2.50	2.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
*Vit./min.premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis (%):						
Crude protein	23.66	22.08	22.07	21.54	20.01	20,00
Crude fibre	4.11	4.89	4.88	5.55	5.86	5.85
Ether Extract	3.44	7.10	7.10	4.32	7.69	7.60
ME (Kcal/kg)	2900.43	2950.50	2944.70	3100.10	3130.78	3125.55

*Bio mix broiler premix supplied the following per 100kg of diet; Vit A, 850,000 I.U., Vit D3, 150,000; Vit E, 1000 I.U, ; Vit K, 1000 mg; Thiamine (B1), 150 mg; Riboflavin (B2), 450mg; Pyridoxine(B6); 300mg; Niacin, 1500mg; Vitamin B12; 1.5mg; Pantothenic acid, 450mg; Folic acid, 60mg; Biotin, 50mg; Chloride; 17,500mg; Anti-oxidant, 125mg; Manganese;5000mg; Zinc, 3000mg; Iron, 2000mg: Copper, 300mg; Iodine, 100mg; Selenium; 20mg: Cobalt;20mg.

Chemical assays

Samples of formulated diets, test ingredient and droppings were oven dried at 60^{o} C for 24 hours and milled (1mm screen), all analyses were performed on dried samples, except for nitrogen of droppings which was determined in fresh samples. Dry matter (DM), ash, crude protein (CP) (N × 6.25), crude fat (EE) and crude fibre (CF) were determined on dry samples according to AOAC (2010) methods. Tannin was determined according to Follin Denis methods (AOAC, 2005). Phytic acid was determined by the colorimetric method. Trypsin inhibitory activity was determined following methods of Kakade *et al*. (1974); hydrocyanic acid was determined by modified alkaline titration method (AOAC, 2010). Total Oxalate content was determined by the procedure of Abaga *et al*. (1968). The metabolizable energy was calculated according to the formula of Pauzenga (1985) as: ME (Kcal/Kg DM) = 37 × % crude protein + 81.8 × % EE + 35.5 × NFE.

Data collection

Feed intake and body weight of birds were determined daily and weekly, respectively. While weight gain, feed conversion ratio and protein efficiency were computed from the values of feed intake, protein intake and live weight. A record of mortality was kept as it occurred. At the 56^{th} day of the experiment, a total of 24 birds were sacrificed for carcass evaluation (that is 8 per treatment & two birds per replicate). They were randomly selected, fasted overnight and their jugular veins severed for prime cut and internal organs indices.

Also, two sets of blood samples (10 ml each) were taken from 3 birds per treatment using a syringe and hypodermic needle into clean bottles. One set was collected into bottles containing anticoagulant – ethylene diamine tetra acetate (EDTA) and used for the determination of haematological parameters while the second set of blood samples was collected into clean sterile bottles devoid of the anticoagulant and used in the determination of serum biochemical parameters. The concentration of red blood cells, white blood cells, packed cell value and haemoglobin were evaluated as described by Ewuola and Egbunike (2008). Other haematological indices including mean corpuscular haemoglobin (MCH), platelets, monocytes, lymphocytes (L), eosinophils, heterophils (H) and H: L ratio were determined using appropriate formulae as described by Jain (1986).

Serum enzymes

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were determined using spectrophotometric method. The serum alkaline phosphate (ALP) activity was determined by the phenolphthalein micro phosphate method.

Serum lipids

The serum lipid determined were cholesterol, triglycerides, high density lipoproteins (HDL) and low-density lipoproteins (LDL). Serum cholesterol was estimated using the mono reagent enzymatic cholesterol colorimetric method (Olorede *et al*., 1996). Serum triglyceride was estimated by colorimetric enzymatic test of glycerol-phosphate oxidase. High density lipoproteins (HDL) was

separated from chylomicrons, very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) by the addition of a precipitating reagent (phosphotunstic acid/magnesium chloride) to serum following centrifugation, the cholesterol content of HDL, fraction remaining in the supernatant was determined by the enzymatic colourimeter method (Olorede *et al*., 1996).

Serum electrolytes

Sodium, potassium, chloride and bicarbonate ions were estimated using the flame photometer. Other electrolytes were determined by standard laboratory methods described in Ochei and Kolhathar (2008).

Determination of meat quality

From the Sacrificed birds, meat samples (two per replicate) from the drumstick and thigh muscles were analysed according to the methods of AOAC (2010) to determine dry matter by oven drying, protein by the Kjeldahl method, fat by Soxhlet fat analysis and ash by muffle furnace.

Nutrient retention

A total of 24 birds per treatment (8 birds per replicate) were randomly selected at the end of 56 days of the experiment and housed in a previously disinfected two- tier wire floor metabolic cages of dimensions 0.7 x 0.6 m floor spacing per pen and a dropping tray inserted for easy collection of faecal droppings. After the initial five days of acclimatization where the birds were fed *ad libitum* with the test ingredient in the morning hours after which the left overs were weighed and sampled. Daily voluntary feed intake was measured and recorded. Their droppings were collected and weighed fresh and later dried using GallenKamp® oven at 80^{o} C for about 48 hours to obtain a constant weight, and milled to pass through a standard 0.02 mm sieve for proximate composition determination (AOAC, 2010).

Cost analysis

Cost analysis using appropriate formulae were used to determine the economics of production based on the current prevailing prices of feedstuffs in Calabar.

$$\text{Feed cost/kg} = \frac{\text{Sum of price of ingredients}}{\text{Kilogram of feed at the time of experiment}}$$

$$\text{Total feed cost} = \frac{\text{Total feed intake} \times \text{Feed cost/kg}}{100}$$

Total cost of feed consumed/Kg (₦) = cost of feed/Kg (₦) × Total feed consumed (Kg/bird)

$$\text{Cost of feed/Kg live weight gain (₦)} = \frac{\text{Total cost of feed consumed/kg (₦)}}{\text{Totall weight gain (g/bird)}}$$

Statistical analysis

Data collected were subjected to one - way analysis of variance using GENSTAT (2011). Where significant differences existed, means were separated using the Duncan New Multiple Range Test (DNMRT) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Proximate, energy and antinutrient composition of diets and sand box seed meal

The result of proximate composition of sand box seed meal and soybean meal is presented in Table 2. The results revealed that sand box seed meal is moderate in crude protein (29.76%) and confirms the high crude protein (42.98%) nature of soybean meal. Soybean meal is one of the best conventional plant protein sources in the world for both humans and livestock. However, sand box seed meal was very high in oil (38.12 %) and crude fibre (15.87 %) than soybean meal with 6.43 % ether extract and 4.99 % crude fibre, respectively. It could therefore be affirmed that sand box seed meal is a good source of energy, fibre and protein in livestock diets. In addition, sand box seed meal is relatively low in phytate (2.55 %), tannins (3.81 %), and saponins (5.07 %) when compared to other leguminous plants like mucuna beans, pigeon pea, and jack bean.

Table 2: Proximate, energy and antinutrient composition of experimental diets and sand box seed meal (SBSM)

	Starter diets			Finisher diets			
Parameter (%)	T_1	T_2	T_3	T_1	T_2	T_3	SBSM
Crude protein	23.20	22.07	22.02	21.31	20.08	20.06	29.70
Crude fibre	4.13	4.96	4.54	5.48	5.79	5.60	18.60
Ether extract	3.70	4.68	4.65	4.53	5.08	5.00	22.01
ME (Kcal/kg)	2898.77	2960.64	2959.80	3105.40	3172.80	3165.52	ND*
Phytate	0.03	0.72	0.65	0.02	0.41	0.39	2.55
Tannin	0.01	0.79	0.77	0.01	0.52	0.46	3.81
Saponins	0.01	1.01	0.97	0.01	0.91	0.89	5.07
Cyanogenic glycosides	0.00	0.21	0.21	0.00	0.13	0.13	0.98

*ND: Not determined

Growth response indicators of birds to sand box seed meal based – diets with or without enzyme

The growth response of broiler chicks to sand box seed meal-based diets with or without Allzyme®Vegpro5X supplementation, is shown in Table 3. The mean daily feed intake (MDFI) of birds over the experimental period differed significantly ($p<0.05$) between the dietary treatments, the birds on the control diet (CD) had significantly ($p<0.05$) higher MDFI than those on SBM diets. Within the two SBM diets, enzyme (Allzyme®Vegpro5X) diet had significantly ($p<0.05$) higher MDFI intake than those without enzyme. The reduction in feed consumption during the starter phase of birds fed SBM-based diets could be related to increase in the energy density of the diets due to the high content of oil in SBM (Table 2), which is in line with the observation of Odunsi and Gbadamosi (2001); Bawa and Odelewo (2007) that monogastric animals are expected to consume less of high energy diet in an attempt not to overshoot their energy requirements. An earlier report by Aduku and Nuhu (1996) also corroborated this view on broilers fed diets containing oil sludge which showed a slight but no significant ($p>0.05$) decrease in feed intake as the dietary oil level increased. Longe and Adekoya (1988) had earlier observed that addition of oil to a diet that is otherwise adequate in energy lowers feed intake. There was significant ($p<0.05$) improvement in feed intake by birds on sand box seed meal-based diets supplemented with Allzyme®Vegpro5X than those on sand box-based diets without the supplement. This improvement in feed intake could be as a result of the enzyme Vegpro 5X, which is one of the exogenous enzymes that helps in breaking down (digestion) and absorption of plant feedstuffs with high fibre and non- starch polysaccharides contents (Bedford and Classen, 1992). Birds are known to consume less of high fibre and high non-starch polysaccharides feed (Nsa *et al*., 2020). However, at the finisher phase there were no significant differences ($p>0.05$) in feed intake between all the treatment means. The similarities in feed intake means there was no inhibition in the consumption of any of the diets. Therefore, any differences observed in other performance parameters could only arise from the utilization of the diets. Each ingredient in all the diets was included at the same level and received the same treatment. However, only the test ingredients (sand box seed meal with or without Allzyme®) Vegpro5X) were used to completely replaced soya bean meal in the diets. Therefore, any differences in the utilization of the diets could be attributed to the influence of sand box seed meal with or without the enzyme as major replacement of soya bean meal in the diets.

The similarity in values of feed consumption at the finisher phase could be due to the fact that at this phase birds needed higher energy level and can tolerate higher fibre levels and anti-nutritional factors in feeds. This observation is in agreement with that of Abeke *et al*. (2011), who stated that birds eat to meet their energy requirements. Mature birds need a higher energy level in their diets than young chicks (Nsa *et al*., 2013). Similar trend was observed for final live weight, daily weight gain, feed conversion ratio and protein efficiency ratio during the starter phase. Birds on the control diets had the best ($p<0.05$) performance, followed by birds on treatment 3 (sand box seed meal-based diet supplemented with enzyme) while the least values were recorded in birds on treatment 2 (sand box seed meal based diet without enzyme).

The poor performance recorded in birds on Treatment 2 may be attributed not only to low intake due to high oil content of the diets but to high fibre level, moderate protein level of the feed and the presence of anti-nutritional factors like tannin and phytate in the diets (Table 2). The presence of anti-nutritional factors has been shown to reduce growth rate of broilers due to reduce protein and specific amino acid utilization (Fanimo *et al*., 2007). Also, phytates are capable of forming complexes with cations resulting in reduced availability of most minerals (Birk and Peri, 1980). According to Fanimo *et al*. (2007) with reduced availability of these nutrients and minerals, animals consuming diets with some probable level of anti-nutritional factors may not be able to meet their nutrients requirements for tissue accretion hence poor growth of the birds fed sand box seed meal. The observed significant ($p<0.05$) better performance observed with birds on sand box seed meal based diets supplemented with enzyme over the birds fed sand box seed meal based diet without enzyme could be attributed to the increase in feed consumption and quality of feed with enzyme. Idahor (2010) made similar observations when he fed broiler finisher birds with various graded levels of sundried cassava peel meal. The improved performance of birds on sand box seed meal-based diets with enzyme over the one without enzyme is a strong indication of the efficacy of the enzyme in assisting in digestion and absorption of sand box seed meal. Bedford and Classen (1992) in their work noted that Allzyme®Vegpro5X assist in digestion and absorption of non-starch polysaccharides and fibre. Sand box seed meal is said to be high in non-starch polysaccharides and fibre contents (Nsa *et al*., 2018). Abeke *et al*. (2011); Ani *et al*. (2010) have all ascertained that enzyme supplementation in fibre causes laxative effect on dietary fibre and enhances a less viscous digesta leading to improved digestion hence improved feed utilization and subsequent growth performance.

At the finisher phase, birds fed sand box seed meal supplemented with enzyme had the same numerical values for final live weight (FLW), daily weight gain (DWG), feed conversion ratio (FCR) and protein intake (PI) with birds on the control diet. This improvement in the nutrient value which encouraged better utilization by the finisher broiler birds could be due to addition of Allzyme® Vegro5X to the sand box meal. Similar observations have been made by Adesehinwa *et al.* (2010) on palm kernel cake-based diets supplemented with Allzyme(R VEGRRO 5X for growing pigs, and found no significant ($p>0.05$)

differences between pigs on the test ingredients and the control diet. The gastro- intestinal tract of birds cannot fully digest or absorb non-starch polysaccharides (Bedford Classen, 1992). Allzyme®Vegro5X is an exogenous enzyme that helps in digestion and absorption of high fibrous and non-starchy polysaccharides feed/feedstuffs which sand box seed meal is noted for (Nsa *et al.,* 2016).

Table 3: Growth performance of broiler birds fed sand box seed meal-based diets with or without Allzyme®Vegpro5X

Chick starter phase:				
	Dietary treatments			
Parameter	T_1(Control)	T_2 (SBSM with enzyme)	T_3 (SBSM without enzyme	SEM
Initial weight (g/bird)	94.67	93.89	94.30	2.87
Final weight (g/bird)	940.22[a]	660.86[c]	828.41[b]	2.47
Daily weight gain (g/bird)	30.20[a]	20.25[c]	26.22[b]	1.09
Daily feed intake (g/bird)	57.70[a]	50.23[c]	56.50[b]	0.65
FCR	1.91[c]	2.48[a]	2.15[b]	0.09
Protein intake	12.69	11.05	12.43	0.64
PER	2.38[a]	1.83[c]	2.11[b]	0.05
Finisher phase:				
Initial weight (g/bird)	988.62	980.00	986.26	6.89
Final weight (g/bird)	2266.70[a]	1765.12[b]	2240.22[a]	9.88
Daily weight gain (g/bird)	45.64[a]	28.04[c]	44.82[a]	3.12
Daily feed intake (g/bird)	131.58	126.79	128.58	9.96
FCR	2.88[b]	4.52[a]	2.89[b]	0.21
Protein intake	26.32	25.36	25.72	1.53
PER	1.73[a]	1.11[b]	1.74[b]	0.09

[abc]Means on the same row with different superscripts are significantly different ($p<0.05$)
SEM: Standard error of mean
PER: Protein efficiency ratio

Carcass characteristics of birds fed sand box seed meal based – diets with or without enzyme

The diets had significant ($p<0.05$) effect on the dressing percentage (Table 4). The birds on sand box meal-based diet had the least ($p<0.05$) dressing percentage while birds on the control and sand box seed meal supplemented with enzyme had statistically ($p>0.05$) similar dressing percentage that were higher than that of sand box seed meal-based diet without enzyme. This is in line with the report of Fanimo *et al*. (2007) who observed that feeds with high level fibre content, lower the metabolizable energy content of the feed which affects feed intake and efficiency which consequently affect the live weight and dressing percentage of the birds. The diet also had significant ($p<0.05$) effect on the head, neck, breast and back cut. With birds on the control diets having higher ($p<0.05$) values of the above parameters followed by birds on sand box-based meal with or without enzyme. This could be due to the effect of reduced feed utilization as a result of high level of sand box meal in the diet. The drum stick, thighs and wings were not significantly ($p>0.05$) affected by the diets. Among the internal organs measured only the gizzard showed significant ($p<0.05$) differences. Birds on sand box seed meal-based diets had the biggest gizzard values followed by birds on sand box seed meal with enzymes. This could be as a result of high fibre in the diet, contributed by sand box seed meal. The abdominal fat values were similar in birds on sand box meal-based diets with or without enzyme but significantly ($p<0.05$) different from that of the control. Sand box seed meal is noted for its high oil content of between 16 and 21.54 % compared to soybean meal of between 7.44 and 8.23 % (Nsa *et al*., 2013). Feeds high in oil encourage abdominal fat deposition in broiler birds (Nsa *et al*., 2012). The weights of small intestine and large intestine values were not significantly ($p>0.05$) different with birds on both sand box based diets with or without enzyme, but significantly higher than that of the control group. This agrees with the findings of Fanimo *et al*. (2007) who fed different levels of cashew nut testa to broiler birds and reported a significant increase in the small and large intestine weight. The increase in the length of gastro-intestinal tract may be attributed to structural adjustment due to high fibre content of the diet. Apart from the meat lipid, other meat composition (Table 5) indicated no significant ($p>0.05$) difference in meat protein, NFE and dry matter and ash. The higher values obtained for meat lipid in meat of birds fed diets 2 and 3 could be as a result of appreciable fat content of oil contributed by sand box meal.

Table 4: Carcass characteristics of broiler chickens fed diets containing sand box seed meal with or without Allzyme®Vegpro5X

Parameter	Dietary treatments T_1 Control	T_2 Sand box seed meal without enzyme	T_3 (Sand box seed meal + enzyme	SEM
Live weight (g/bird)	2201.50	1880.44	2199.30	11.02
Dressed weight (g/bird)	1730.82	1412.40	1577.78	8.66
Dressing percentage	78.62[a]	75.11[b]	71.74[a]	3.04
Relative weight of parts (% LW):				
Head	3.01	2.68	2.99	0.11
Neck	3.66[a]	3.39[c]	3.51[b]	0.13
Breast	20.81[a]	20.03[c]	20.55[b]	1.13
Back	12.81[a]	10.69[c]	12.00[b]	1.01
Drumstick	9.47	7.93	8.99	0.99
Thighs	11.05	10.39	11.02	0.16
Shanks	3.99	3.87	3.88	0.09
Wings	8.95	8.11	8.67	0.08
Relative weight of organs (% LW)				
Heart	0.30	0.40	0.39	0.01
Kidney	0.03	0.03	0.04	0.00
Lungs	0.48	0.44	0.44	0.00
Liver	1.42	1.43	1.33	0.01
Gizzard	1.08[c]	1.30[a]	1.19[b]	0.03
Abdominal fat	0.13[b]	0.29[a]	0.23[a]	0.04
Small intestine	2.30	3.41[a]	3.39[a]	0.06
Large intestine	1.11[b]	1.20[a]	1.19[a]	0.03

[abc]Means on the same row with different superscripts differ significantly ($p< 0.05$)

Table 5: Meat nutrient composition of broiler chickens fed sand box seed meal based - diets with or without Enzyme (finisher phase)

Parameter (%)	Dietary treatments T_1 Control	T_2 Sand box seed meal without enzyme	T_3 (Sand box seed meal + enzyme	SEM
Dry matter	96.08	95.96	95.82	7.9
Crude protein	32.40	31.89	31.93	1.0
Lipid	8.60[b]	9.98[a]	9.91[a]	0.8
Ash	9.03	9.10	9.08	0.6
Nitrogen free extract	50.04	50.11	50.07	1.6

Nutrient retention by birds fed sand box seed meal-based diets with or without enzyme

The retention of nutrients of the diets by broiler birds is presented in Table 6. The digestible dry matter (DM), crude protein (CP) and crude fibre (CF) contents of sand box meal-based diets without enzyme had poorer ($p<0.05$) nutrient retention values than the controlled diets and sand box meal supplemented with enzymes. This observation is a clear indication that sand box meal is poorly utilized by poultry birds when compared to soybean meal. Similar report by Nsa *et al*. (2019) showed poor nutrient retention by birds fed high level of sand box meal in their diets. The same reason of high content of fibre and non-starch polysaccharides could be adduced here. This result is in agreement with findings of Clasen *et al*. (2004) who reported an inverse relationship between dietary fibre digestibility and bio-availability of nutrients. The sandbox seed meal-based diets supplemented with enzyme had similar ($p>0.05$) retention values of the nutrients with the control diet. The improvement in nutrient

retention of birds on sand box seed meal supplemented with enzyme over birds on sand box seed meal-based diets without enzyme could be due to the facts that enzymes in feeds help in breaking down of the non-starch polysaccharides (NSPs) and fibre content of feed. They also enhance a less viscous digesta leading to improved digestion (Edache *et al*., 2018). This ultimately leads to improved nutrient retention.

Table 6: Nutrient retention in broiler chickens fed sand box seed meal based - diets with or without Enzyme (finisher phase)

Parameter (%)	Dietary treatments T_1 Control	T_2 Sand box seed meal without enzyme	T_3 Sand box seed meal + enzyme
Dry matter	61.62[a]	58.06[b]	61.47[a]
Crude Protein	47.41[a]	39.69[b]	45.91[a]
Crude fibre	21.69[a]	18.41[b]	21.72[a]
Ether extract	75.50	79.33	77.53
Nitrogen -free extract	79.05	75.10	78.57

Economics of production of birds fed sand box seed meal-based diets with or without enzyme

The production cost per kilogramme diet (Table 7) stood at ₦240.88, ₦2110.43 and ₦233.76 for treatment groups 1, 2 and 3, respectively. The least cost was obtained in birds fed sand box seed meal-based diets. This is because sand box seed is a non - conventional feedstuff and the only cost incurred was the processing method used in extracting the meal. The higher cost of treatment 2 is the cost of the enzyme. While treatment 1 incurred the highest cost, this could be traced to the cost of soybean meal, which the test ingredient which replaced 1 kg of soybean meal at ₦300.00 while that of sand box meal without enzyme and sand box meal with enzyme were ₦16.00 and ₦23.09, respectively. The production cost (₦) of a unit (kg) weight gain by birds was inversely related to the cost (₦) of a unit per kg diet, as birds on treatment 3 had the best ($p<0.05$) cost per kg weight gain followed by the control group. Treatment 2 gave the highest cost of producing per kg weigh gain.

Table 7: Economic evaluation of broiler chickens fed sand box seed meal-based diets with or without enzyme (Finisher phase)

Parameter	Dietary treatments T_1 Control	T_2 Sand box seed meal without enzyme	T_3 (Sand box seed meal + enzyme	SEM
Total weight gain (Kg/bird)	2.12	1.35	1.99	0.05
Total feed consumed /Kg(₦)	5.30	4.96	5.18	0.56
Cost of feed /Kg(₦)	250.55	235.60	240.06	4.65
Total cost of feed consumed (₦)	1327.92[a]	1168.58[c]	1227.97[b]	11.88
Cost of feed /Kg Live weight gain (₦)	626.38[c]	865.61[a]	617.07[b]	1.99

[abc]Means on the same row with different superscripts are significantly different ($p<0.05$)
SEM: Standard error of mean

Blood characteristics of broiler chickens fed sand box meal-based diets with or without enzyme

In this study, the result of haematological parameters (Table 8) of broiler chickens fed sand box meal based diets supplemented with or without Allzyme(R) Vegpro 5× recorded significant differences ($p<0.05$) between dietary treatments for white blood cell counts (WBCs), lymphocytes, red blood cell counts (RBCs), haemoglobin concentration, haematocrit, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). However, the mean corpuscular haemoglobin concentration (MCHC) and platelets were not significantly influenced by dietary treatments. Nutrition has a profound effect on the blood parameters of animals (Schalm, 1986). Dietary content affects the blood profile of healthy animals (Yeong, 1999). Haematological investigations have been extensively explored to distinguish normal state from nutritional stress in farm animals (Aro *et al*., 2013).

The range of values for WBC (8.77 - 9.26 $\times 10^3$/L) was slightly lower than the normal range (12 - 30 $\times 10^3$/L) for broiler chickens (Bounous and Stedman, 2000). This implies that the anti-nutrients in the sand box seed meal with enzyme had slightly adverse

effect on the immune status of the birds. This is because high WBC counts are associated with microbial infection or the presence of foreign bodies or antigens in the circulating system. The lymphocyte counts were slightly lower in sand box meal based-diet with enzyme compared with the control; implying that sand box seed meal- based diets had depressing effect on the pathological conditions in the blood, that would have helped in combating possible infection. The haematocrit or PCV values (9.00 - 11.46 %) obtained in this study were lower than the normal range (22-35%) for broiler chickens reported by Bounous and Stedman (2000). This implies that the PVC values were seriously affected by dietary effect. This agrees with the report of Banergee *et al*. (2000) that packed cell volume is mostly affected by dietary treatments. The result for red blood cell counts (1.93-2.41 × 10^6/µl) was within the normal RBC range (2.5-3.5 × 10^6/µl); suggesting no adverse effect of sand box seed meal- based diets on erythropoiesis. The same trend was recorded in the haemoglobin concentration. The range of 9.00 - 11.46 g/dl obtained in this study was within the normal reference values (7-13 g/dl) for broiler chickens (Bounous and Stedman, 2000). This suggests that sand box seed meal based-diets were nutritionally adequate to meet the protein needs of the birds, since the haemoglobin concentration decreases in animals on lower protein diets and during parasitic infections of the liver (Lindsay, 1997). There is evidence in literature that haematological indices of livestock suggest their physiological disposition to the plane of nutrition (Madubuike and Ekenyem, 2006). In this study, reduction in haemoglobin concentration is indicative of low protein intake or mild anaemia (Lindsay, 1977), even though the birds did not manifest any obvious symptom.

The MCV, MCH and MCHC reduced significantly in values in the sand box seed meal diets with or without enzyme compared to the control diet. However, the range of MCV (120.44 - 128.61 fl) obtained in this sturdy was within the recommended range (90 - 140 fl) for healthy birds reported by Bounous and Stedman (2000). While the ranges of MCH (54.23 - 61.49 pg/dl) and MCHC (41.31 - 44.82g/dl) recorded in this study were higher than the normal ranges (33 - 47 pg/dl) and (26 – 35 g/dl), respectively, reported by Bounous and Stedman (2000). This suggests that the blood of the broiler chickens may have an appreciable oxygen-carrying capacity; which showed that nutrient transport by the blood might not impaired be by diets containing sand box seed meal with or without the enzyme.

Serum biochemical indices (Table 9) except total protein and bicarbonates recorded in this study showed significant ($p<0.05$) differences between dietary treatments. The serum protein and albumin decreased across dietary treatments, implying and decreased serum activity in broiler chickens fed diets with sand box seed meal with or without the enzyme; this suggest a decrease in protein synthesis and lipid metabolism (Rose *et al*., 2001). Serum albumin was lowest in the birds fed sand box seed meal enriched with Allzyme; suggesting poor immune response and insufficient antibody production in birds of that group. The serum cholesterol, HDL, LDL, Cl^-, AST, ALT and Na^+ in this study recorded fluctuated trends across dietary treatments that could not be ascribed to dietary treatment effect. Serum total protein consists of albumin and globulin; a change in nutritional status and malnutrition are revealed in total protein values (Allison, 1995).

Table 8: Haematological characteristics of broiler birds fed sand box seed meal based – diets with or without enzymes

Parameter	T_1 Control	T_2 Sand box seed meal without enzyme	T_3 (Sand box seed meal + enzyme	SEM
WBC (×10^3/L)	8.77^b	9.26^a	8.94^b	2.96
Lymphocytes (%)	79.89^a	60.00^a	64.62^b	4.01
RBC (×10^6/L)	2.41^a	1.93^b	2.24^a	0.06
Haemoglobin (g/dl)	1.46^b	9.44^c	19.00^a	1.24
Haematocrit (%)	39.40^a	11.46^b	36.06^a	3.00
MCV (fl)	128.61^a	120.44^b	121.25^b	11.15
MCH (pg)	61.49^a	54.23^{ab}	57.06^b	2.71
MCHC (%)	44.82	41.31	42.60	1.99
Platelets (×10^6/L)	13.11	15.55	13.20	0.86

^{abc}Means on the same row with different superscripts are significantly different ($p<0.05$)
SEM: Standard error of mean

Table 9: Serum biochemical indices of broiler birds fed sand box seed meal based – diets with or without enzymes

Parameter	T_1 Control	T_2 Sand box seed meal without enzyme	T_3 (Sand box seed meal + enzyme	SEM
Cl^-(mmol/L)	102.02^b	140.13^a	103.45^b	9.44
K^+(mmol/L)	5.60^b	6.72^a	6.71^a	0.32
Na^+(mmol/L)	138.81	145.66		13.63
AST (U/L)	138.81^b	145.66^a	140.91^{ab}	8.94
ALT (U/L)	153.50^a	120.96^b	125.22^b	0.03
ALP (U/L)	11.03^a	1.15^b	1.13^b	5.60
HDL (mg/dl)	79.41^a	62.40^c	68.00^b	0.18
T. CHOL. (mg/dl)	4.69^b	5.17^a	4.82^b	0.03
Total protein (g/dl)	40.08	40.09	39.21	3.11
Albumin (g/dl)	30.07^a	28.87^b	20.58^c	1.88
Globulin (g/dl)	10.11^c	11.22^b	18.63^a	1.01
HCO^-_3 (g/dl)	22.55	22.61	22.46	1.89

^{abc}Means on the same row with different superscripts are significantly different ($p<0.05$)
SEM: Standard error of mean

CONCLUSION

The feeding cost of broiler birds can thus be reduced and the profit margin increased with the replacement of soybean meal with sand box seed meal, which is a relatively cheaper protein feedstuff in diets of broiler birds. The study showed no adverse effect on growth performance but slightly adverse effect on blood chemistry of broiler chickens. Hence, the study concluded that sand box seed meal supplemented with enzyme (Allzyme®Vegpro5X) has tremendous potential in boosting growth performance as an alternate protein source to conventional plant source (soya bean meal) for broiler birds in the face of expensive and scarce soybean meal.

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