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Effect of multi-enzymes and probiotics on growth and health status of cockerel chickens

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***Target Audience:** Animal Scientists, Feed millers, Poultry Farmers and Animal Nutritionists*

Abstract

This experiment was conducted to evaluate the effect of multi-enzymes and probiotics on growth performance and health status of cockerel chickens. A total of One hundred and twenty (120) cockerel chicks were allotted to four (4) dietary treatments with three (3) replicates and each having ten (10) birds in a completely randomized design (CRD). The treatment (T) diets were formulated; T1: control diet, maize based diet only, T2: maize based diet with multi enzyme, T3: maize based diet with probiotics, T4: maize based diet with multi enzyme and probiotics in combination. The diets and clean water were provided ad libitum for the eight weeks of the experiment. Data were subjected to Analysis of Variance (ANOVA) and significant differences among treatment means were compared using the Duncan Multiple Range Test. Addition of multi-enzyme and probiotics singly or in combination improved (P<0.05) the final weight gain and feed intake of cockerel chicks. The inclusion of multi enzyme and probiotics increased the albumin levels in cockerel chickens and improve liver function. It was concluded that the use of multi-enzymes and probiotics singly or in combination can improve the growth and health status of cockerel chickens.

***Keywords:** Feed conversion ratio, nutrient utilization, performance, synergy*

Description of Problem

Poultry is one of the fastest growing industries within the agricultural sector, with huge interest in animal nutrition, research and development focused on improving health, disease resistance and productivity (1).

Cockerel production is easier than other poultry species because the bird survives particularly in the rural area where infrastructural facilities are not available (2). Cockerel meat is one of the best healthier chicken meats consumed by many which has less fat, taste better than broiler meat and less expensive compared to broilers (3). The body system is not completely efficient in the discharge of its metabolic functions, there is therefore need for external intervention by enzymes, hormones and antibiotics. Recently, application of enzymes and probiotics feed supplements to poultry diets has been increasingly focused with little information on their effect in cockerel chickens (4). Enzyme are one of the many types of protein in biological systems. Their essential characteristic is to catalyze the rate of a reaction but is not themselves altered by it. They are involved in all anabolic and catabolic pathways of digestion and metabolism. The incorporation of enzyme

combination containing Xylanase, Amylase and Protease (XAP) can increase the nutrient digestibility of feed and can thereby enhance average gain and improve feed efficiency in chickens. (4).

Probiotics are single or mixed cultured of living microorganisms which when administrated in adequate numbers apply health benefits for the host by improving intestinal microbial balance; increase of colonization resistance against pathogens and improving the immune response (5). Research have shown a positive effect of exogenous enzyme combination (Xylanase, Amylase and Protease) on broilers growth performance and nutrient utilization. Xylanase targets hemicellulose in cell wall and releases entrapped starch and protein while amylase and protease assist by hydrolyzing these substrates of nutrients by releasing enzymes and boosting endogenous enzymes. Similarly, supplementation of probiotics, beneficially modulates gut microbiota, gut barrier function and competitively excludes pathogenic bacteria (6). The use of probiotics along with multi-enzymes can produce more benefit due to additive or synergistic effect by improving growth performance, gut health and digestion (7,8).

Broiler chickens provide most of the world production and consumption of poultry meat. This has been achieved because of the rapid growth due to genetic selection and researches on nutrition to meet their nutritional requirement. Conversely, little research has been performed on the nutritional factors of cockerels. At present, most diets formulated are uneconomical for the producers of cockerels. Cockerel producers are under pressure to maximize value from their feed and minimize feed cost with optimal performance hence prompt the research work.

This research focuses on evaluating the synergetic effect of multi-enzymes of xylanase, amylase and protease (XAP) and probiotics (PRO) on growth and health status of Cockerel chickens.

Materials and methods

Study location

The experiment was conducted at the Poultry research unit, National Veterinary Research Institute, Vom, Plateau State, Nigeria. The study area is located on Latitude 09° 44′ N and Longitude 08°45' E with a physical feature of rocky granites of old volcanoes at an altitude of 1285 m above sea level with a mean annual rainfall ranging between 1300 to 1500 mm and average daily temperature ranges between 17 ºC to 28.6 ºC. The wet season extends from late April to middle October and relative humidity ranges from 22% in January to 78% in July/August. Mean monthly sunshine hours ranges from 177 - 288.3 (9).

Experimental diets

Four (4) treatment diets were formulated as shown in Table 1.

T1: control diet, maize based diet only (MBD)

T2: maize based diet with multi enzyme (MBD +XAP)

T3: maize based diet with probiotics. (MBD + PRO)

T4: maize based diet with multi enzyme and probiotics in combination. (MBD+XAP+PRO)

Experimental animal

One hundred and twenty (120) day - old white cockerel chicks were allotted to four (4) dietary treatments with three (3) replicates and each having ten (10) birds in a completely randomized design (CRD). The birds were raised on a deep litter system with wood shavings serving as a source of litter. Feed and water were provided *ad libitum*.

Immediately after arrival, all the chicks were provided with glucose through drinking water for a day. Vitalyte® was continued for 3 days along with drinking water. Heat and light were provided throughout the brooding period using kerosene stoves and electric bulbs. Routine vaccines (Gumboro and Newcastle) were also administered.

Table 1. Composition of experimental diets

	T1	T2	T3	T4
Feed ingredients (kg)	**MBD**	**MBD+XAP**	**MBD+PRO**	**MBD+XAP +PRO**
Maize	54.00	54.00	54.00	54.00
Soya bean meal	16.00	16.00	16.00	16.00
Groundnut cake	14.00	14.00	14.00	14.00
Maize offal	12.00	12.00	12.00	12.00
Bone meal	3.00	3.00	3.00	3.00
Limestone	0.25	0.25	0.25	0.25
Common salt	0.30	0.30	0.30	0.30
Vit/Mineral Premix*	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.20	0.20
Axtra® XAP 101 TPT enzyme +	0.00	0.02	0.00	0.02
YungStrong® probiotics +	0.00	0.00	0.10	0.10
TOTAL	100.00	100.00	100.00	100.00
Calculated Analysis				
ME (Kcal/Kg DM)	2908	2908	2908	2908
Crude protein (%)	20.07	20.07	20.07	20.07
Crude fibre (%)	4.79	4.79	4.79	4.79
Ether extract (%)	3.23	3.23	3.23	3.23
Calcium (%)	1.24	1.24	1.24	1.24
Phosphorus (%)	0.88	0.88	0.88	0.88
Lysine (%)	1.07	1.07	1.07	1.07
Methionine (%)	0.49	0.49	0.49	0.49

*Biomix broiler starter premix included per Kg diet: Vit A, 1,000 I.U; Vit D, 2000 IU; Vit E, 5.0; Vit K, 2mg; Vit B1, 1.8 mg; Vit B2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 0.5mg; Vitamin B6 0.30 mg; Vit B12, 0.15mg; Folic acid, 0.75mg; Biotin, 0.6mg; chlorine chloride, 300mg; Iodine, 1mg; iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg. ME= Metabolizable Energy, +Feed additves exclusive of 100kg diet. XAP:Xylanase Amylase Protease, PRO: Probiotics

Performance study

Initial weights of the birds were taken at the beginning of the feeding trial. Feed intake and body weight changes were monitored on weekly basis. Feed/gain ratio and cost per Kg gain were computed from the feed intake and weight gain data. Protein Efficiency Ratio (PER) was computed from the weight gain and protein intake of the birds. Mortality was recorded as it occurred.

Serum biochemical tests

At the 8th week, blood samples were collected from one bird randomly selected from each replicate per treatment via the jugular vein section and placed in plain test tubes without an anticoagulant (2.5 ml). The plain tubes were centrifuged at 1500 x g at 4 °C for 10 min to obtain sera. The biomarkers of hepatic function such as serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) albumin (ALB), total protein (TP) total bilirubin, direct bilirubin and renal function test; urea and creatine and electrolytes; sodium ($Na^{+)}$ and potassium

(K^+) were determined in the serum using determined using a commercial assay kit (Agape Diagnostics, Switzerland GmbH) according to the manufacturer's specification.

Economics of broiler chicken production

The economics of production of broiler chickens was calculated by calculating other expenses which is adding the cost of chicks, cost of enzymes and probiotics, vitalyte, vaccines, charcoal, kerosene and miscellaneous expenditures. Total expenses/bird was calculated by summing up the feeding cost and other expenses together. The average yield cost was calculated by multiplying the average final weight by cost of chicken/kg while, the net profit was calculated by subtracting the total expenses from the average yield cost.

Statistical analysis

Data obtained in the experiment was statistically analyzed using the General Linear Model Procedure of SAS (SAS, 2002) and significant difference between treatments means was separated by Tukey Range Test.

Results and discussion

The performance of cockerel birds fed maize based diet supplemented with multi-enzyme and probiotics is presented in Table 2. Addition of multi enzyme and probiotics in combination (T4) improved the final weight and weight gain of cockerel chicks ($p<0.05$). Cockerel chicks fed maize based diets with probiotics inclusion and multi enzyme and probiotics in combination (T3 and T4) had lowest feed consumption ($p> 0.05$) compared to chicks fed maize based diet alone and maize based diet with enzyme inclusion (T1 and T2). Cockerel chickens fed MBD+XAP, MBD+PRO and MBD+XAP+PRO (T2, T3 and T4) had better feed conversion ratio compared to chickens fed maize based diet without feed additives (T1). Feed cost per kilogram weight gain showed that MBD+XAP+PRO had the least cost of production compared to other treatment diets.

Table 2: Performance of cockerel chickens fed maize based diet supplemented with multi- enzyme and probiotics

Parameters	T1 MBD	T2 MBD+XAP	T3 MBD+PRO	T4 MBD+XAP+PRO	SEM
Initial weight (g/bird)	37.73	37.53	37.53	37.46	1.12
Final weight (g/bird)	867.00^{b}	921.33^{b}	857.00^{b}	1103.66^{a}	77.87
Weight gain (g/bird)	829.26^{b}	883.80^{b}	819.46^{b}	1066.20^{a}	77.97
Feed intake (g/bird)	2382.02^{a}	2256.09^{a}	2042.70^{b}	2101.81^{b}	99.84
Feed conversion ratio	2.99^{c}	2.57^{b}	2.50^{b}	2.02^{a}	0.19
Feed cost/Kg gain (₦/Kg)	304.65	278.296	273.08	227.36	22.09
PER	1.63	1.84	1.90	2.40	0.20
Mortality (%)	10.80	6.66	6.66	0.00	3.72

^{a,b,c} Means with different superscripts on the same row are significantly ($P<0.05$) different, SEM: Standard error of means, MBD: Maize based diet, XAP: Axtra® XAP 101 TPT enzyme, PRO: PER: Protein efficiency ratio.

Cockerel chickens fed maize based diets with feed additives had higher values of protein efficiency ratio compared to chicks fed diet without feed additives.

Addition of multi enzyme and probiotics in combination in treatment 4 (MBD+XAP+PRO) had a significant ($p<0.05$) effect on final weight and weight gain of cockerel birds. The noticeable improvement in final weight and weight gain of cockerel birds could be attributed to the combination of multi-enzyme and probiotics. The combination of this feed additives may have increased the availability of nutrients and nutrient absorption. This result is supported by the findings of (10 and 8) who reported that addition of feed additive in broiler feed greatly enhanced the growth rate and weight gain of chicks.

The decrease in feed intake noticed in treatments 3 and 4 could be as a result of the presence of probiotics which is in line with findings of (11) who reported that feed intake in broiler chickens was decreased by supplementation of probiotics (*Bacillus Subtilis*). In contrast to these findings, (12) reported higher feed intake in broiler chickens which were fed with diet supplemented with probiotics (Probiotics Enhancer, USA). The difference in the findings might be related to the type of birds, probiotic strain and dose rate. The results of feed conversion ratio, feed cost per kilogram gain and protein efficiency ratio also showed that exogenous enzymes and probiotics, singly or in combination had a positive effect on the birds. This indicated that the values of feed conversion ratio could be attributed to the effect of enzyme and probiotics supplementation which helps to enhance the bioavailability of nutrients present in the diets, presence of beneficial micro- organism helping to improve the gut system of chickens and improved digestion and absorption of nutrients leading to more meat per kilogram of feed (13).

The effect of maize-based diet supplemented with multi-enzyme and probiotics on serum clinical chemistry parameters is presented in table 3. The albumin (ALB) concentration in chickens fed MBD+XAP+PRO was ($p<0.05$) lower compared to chickens fed treatment diet MBD+XAP and MBD+PRO but was comparable with chickens fed MBD alone. Cockerel chickens fed MBD+XAP and MBD+PRO had ($p<0.05$) reduced concentration of direct bilirubin (DB) compared with the control (T1). The chickens fed MBD+PRO had ($p< 0.05$) decrease in urea concentration compared to groups fed MBD alone, MBD+PRO and MBD+XAP+PRO. The electrolyte, sodium ion was highest (229.34) in chickens fed MBD+XAP while those chickens fed MBD+PRO had the least (168.14) value.

The reduced AST and DB in T2 and T3 indicates the supplementation of feed with XAP or PRO improved liver function. Lower than normal bilirubin levels are usually not a concern and the DB is more valuable than total bilirubin (TB) for predicting prognosis in patients (14). Furthermore, the inclusion of XAP in diet improved liver and kidney function, evidenced by the low AST and DB levels in the sera. The increase in albumin and creatinine levels in chickens diet supplemented with XAP or PRO (T2 and T3) suggest that the enzymes and probiotics improved albumin availability possibly via increased synthesis, protein degradation influenced by protease and probiotics. The findings of the current study agree with previous reports which found increased creatinine in broiler chickens fed low protein diets (15).

Table 3: Effect of maize-based diet supplemented with multi-enzyme and probiotics on serum clinical chemistry

Parameters	T1 MBD	T2 MBD+XAP	T3 MBD+PRO	T4 MBD+XAP+ PRO	SEM
Aspartate Amino Transferase (U/L)	135.16^{a}	89.58^{b}	109.12^{a}	111.08^{a}	18.74
Alanine Amino Transferase (U/L)	15.50	15.00	17.00	16.33	1.24
Alkaline Phosphate (U/L)	92.12	77.00	99.75	103.58	17.71
Total Protein (g/dl)	42.84	44.75	46.27	34.51	4.51
Albumin (g/dl)	20.87^{bc}	26.55^{a}	25.53^{ab}	20.02^{c}	1.18
Total Bilirubin (mg/dl)	2.55	1.65	2.21	1.99	0.25
Direct Bilirubin (mg/dl)	1.77^{a}	0.98^{b}	1.08^{b}	1.18^{ab}	0.13
Urea (mg/dl)	40.89^{a}	38.77^{a}	34.51^{b}	37.73^{ab}	1.43
Creatinine (mmol/l)	0.34^{b}	1.07^{a}	0.39^{b}	0.40^{b}	0.20
Na^{+} (mEq/L)	196.51^{ab}	229.34^{a}	168.14^{b}	180.31^{ab}	11.57
K^{+} (mEq/L)	25.63	29.78	24.33	24.14	2.49

MBD: Maize based diet, XAP: Axtra® XAP 101 TPT enzyme, PRO: SEM: Standard error of means, Na^{+}: sodium ion, K^{+}: Potassium ion.

Table 4: Effect of multi-enzyme and probiotics combination in maize based diets on economics of production

Parameters	T1 MBD	T2 MBD+XAP	T3 MBD+PRO	T4 MBD+XAP+PRO
Feed intake (Kg/bird)	2.38	2.25	2.04	2.10
Feeding cost (₦/bird)	249.90	243.76	222.36	235.91
Other expenses (₦ /bird)	450.00	450.00	450.00	450.00
Total expenses (₦/bird)	699.90	693.76	672.36	685.91
Average final weight (Kg/bird)	0.87	0.92	0.86	1.10
Cost of chicken/Kg (₦)	1000	1000	1000	1000
Average yield cost (₦/bird)	870	920	860	1100
Net profit (₦/bird)	170.10	226.24	187.64	414.09
Profit above maize control diet (₦)	0.00	56.14	17.54	243.99

MBD: Maize based diet, XAP: Axtra® XAP 101 TPT enzyme, PRO: Yung Strong probiotics

The result for economics of production of cockerel chickens fed maize based diet supplemented with multi-enzyme and probiotics is shown in Table 4. The results showed that chickens fed MBD+XAP, MBD+PRO and MBD+XAP+PRO had lower feed intake compared to birds that fed MBD alone. In monetary value, the cost of feeding was least in birds fed MBD+PRO and highest in birds fed MBD alone. The average yield cost was least for birds fed MBD+PRO and highest in birds fed MBD+XAP+PRO. The net profit of cockerel chicken showed that birds fed maize based diet with feed additives inclusion had a better net profit compared to birds fed maize based diet without feed additives.

The performance of the cockerel birds considered in economics of production were favorable and impressive with

inclusion of feed additives (multi-enzyme, probiotics and combination of multi-enzyme and probiotics) in maize based diet. These results agreed well with those of (9) who reported that inclusion of enzymes and eubiotics in sorghum-based diet for broiler chicken improved economics of production. The result also is in line with (16) who reported that addition of commercial exogenous enzymes offers potential to reduce diet cost commensurate with enhanced production.

Conclusions and Application

The following conclusion were drawn from the result obtained in this study:

1. The inclusion of multi enzyme and probiotics singly or in combination improved body weight, feed intake, feed conversion ratio, and protein efficiency ratio of cockerel chickens.
2. The application of multi enzyme and probiotics had no adverse effect on the health status of cockerel chickens.
3. The cost of producing cockerel chickens fed maize based diet with feed additives was reduced compared with chickens fed only maize based diet.
4. The findings, suggest that Axtra® XAP 101 TPT enzyme and YungStrong® probiotics can be added at 2.0% and 1.0% in the diet to improve the growth performance and health status of cockerel chickens.

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