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Comparative Study of the Effects of Probiotic and Commercial Enzyme on Growth Rate, Haematology and Serum Biochemistry of Broiler Chicken

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Abstract

The study investigated the combined and individual effects of probiotic (*Saccharomyces cerevisiae*) and a commercial enzyme (Zyme®) on weight gain, haematology and serum biochemistry of broiler chicken. Eighty day–old broiler chicks were randomly divided into 4 groups (P1-P4) of 20 birds each. Each group was subdivided into 5 replicates of 4 birds each. P1 had no probiotic and no enzymes (control). P2 had enzymes in water (0.02 ml/lit) but no probiotic. P3 had enzymes in their water (0.02 ml/lit) and probiotic in their feed (0.8 g/kg). P4 had probiotic in their feed (0.8 g/kg) but no enzymes in their water. The results showed that birds in group P4 had significantly ($P \leq 0.05$) higher final mean weight (2.51 ± 0.05 kg/bird) followed by P3 (2.43 ± 0.05 kg/bird) while P1 (the control) had the least final mean weight (2.31 ± 0.02 kg/bird). There was a significant difference ($P<0.05$) in Eosinophil levels in P3 (probiotic and enzyme) and P4 (probiotic only). Serum cholesterol significantly ($P \leq 0.05$) decreased while total proteins were higher in probiotic treated groups (P3 and P4). P3 (probiotic + enzyme) gained less weight than P4 (Probiotic only), supplementation in the feed with probiotic alone is recommended for improved broiler production in this environment using this type of diet.

Keywords: Broilers; Feed; Probiotic; Enzyme; Weight gain

Introduction

Poultry production is very important in the economy and supply of animal protein which is grossly insufficient in developing countries. Smith [1] suggested that supply of poultry products in poorer countries could be rapidly expanded to meet the need for animal protein. This is technically possible because poultry are able to adapt to most areas of the world (including the tropics), have rapid generation time and a high rate of productivity. However, Nutrition and diseases have been identified as the major limiting factors in poultry rearing [2]. In Nigeria, the cost of feed alone account for 70-80% of the total cost of production [3]. Nworgu [4], earlier reported that high cost of feed is the major problem of poultry farmers in Nigeria as feed accounts for 65-80% of the total production.

The high cost of feed ingredients has caused farmers to feed poultry with poor quality agro by-products such as palm kernel cake and rice husk which reduces the efficiency of feed utilization due to high fiber contents [5].

Research workers are therefore challenged to identify possible feed additives that help livestock to utilize these fibrous feed stuffs. Feed supplementation is an important aspect of poultry nutrition, since it has been shown to increase the efficiency of feed utilization [6,7].

Consequent on the prohibition of the sub therapeutic use of antibiotics as growth promoters in poultry feeds due to their undesirable effects such as the residues in meat products and development of antibiotic resistant bacteria populations, research efforts in probiotics and enzyme supplementation has gained much attention.

Advances in the field of enzyme technology have brought benefits to the pig and poultry industries for almost 10 years. By adding enzymes to feed, the majority of problems traditionally associated with cereal-based rations can be alleviated. Use of enzymes to treat feeds such as corn or sorghum and soy bean meal is a more recent concept. In corn and sorghum, as with the grain-based diets, xylase enzymes are effective in degrading the fibrous cell walls of feed grains and releasing nutrients previously inaccessible to the animal.

Furthermore, recent studies have revealed that in a normal bird, starch digestion may not be completed due to the absence of sucrase enzyme [8]. Sucrase enzymes are available in *Saccharomyces cerevisiae* [9]. Major part of poultry feed contains considerable amounts of non-starch polysaccharides (NSPs) and phytates. These NSPs and phytates are anti-nutritive factors in poultry feed. The ingestion of soluble NSPs like b-glucans increases the digester viscosity 0020 [10] in broiler chicken. Phytate or phytic acid is a naturally occurring organic complex found in plants and 60-80% of phosphorus found in the cereal grains and oilseeds exists as phytate. Probiotics such as *Saccharomyces cerevisiae* are known to contain phytase enzyme. Paul [11] defined enzymes as proteins that facilitate specific chemical reactions. Some enzymes commonly used as feed supplements include, Phytase; which are known to degrade phytate phosphorus (anti nutritive factor) found in the feed stuff to improve phosphorus utilization as well as other minerals and proteins. This will in turn decrease bacterial proliferation in small intestine, decrease fecal moisture, increase feed intake and utilization and increase nutrient sparing [7]. Others are non- Starch Polysaccharides Enzyme (NSPases); which degrade non-starch polysaccharides (anti nutritive factor) that function by decreasing the viscosity of the digestion, which in turn increase cell availability for nutrients and consequently increase cell permeability and animal performance [7]. Other important enzymes are Amylases, Proteases, Xylanases, Mannanases, and Cellulases among others. These enzymes increase feed intake, improve bird performance, increase feed utilization and increase nutritional diffusion [7]. Probiotic is

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defined as a live microbial feed supplements which beneficially affects the host animal by improving the intestinal microbial balance [12]. In poultry nutrition, probiotic organism which have shown to have beneficial effects on broilers are; *Lactobacillus, Streptococcus, Bacillus, Saccharomyces, Candida, and Aspergillus*. The beneficial effects of *S. cerevisiae* are attributed to the fact that it is naturally rich sources of protein, minerals and B-complex vitamin [13]. The advantage of these growth promoters over traditional antibiotics growth promoters are; no withdrawal time, no residue effects and no causes of microbial mutation [14].

For a normal cell to function properly it requires constant supply of raw materials such as nutrients, hormones and electrolyte; at the same time have their undesirable by-products removed from them. Blood is therefore the medium of transportation of all these substances in the body. Blood contains biochemical components, such as protein (albumin, globulins and fibrinogens), enzymes (Aspertate Amino Transferase, Alanine Amino Transferase, Alkaline Phosphatase among others), glucose, lipids, hormones and minerals. Haematological determinations are important procedures in diagnosis of several diseases such as liver damage and dysfunction as they also provide reliable pathology and biochemistry [15]. Nutritional supplements should be geared towards producing positive effects on the animal, promoting its health status and productive function as well as minimizing any side effects.

Statement of the problem

- Poultry feeds are costly.
- Farmers always want to improve the efficiency of feed utilization by their birds.
- Poultry feedstuffs contain some anti-nutritive factors which decreases the efficiency of their utilization.
- Some commercially available feed additives are not effective in our environment.

Objective of the study

- To compare the effects of probiotic and commercial enzymes supplementation on weight gain in broilers chicken
- To determine the combined effects of probiotic and commercial enzymes on weight gain.
- To evaluate the effects of probiotic and commercial enzyme supplementations on haematology and blood biochemistry of broiler chicken (Table 1).

s/n	Ingredients	%	CP%	ME(Kcal/kg)
1	Maize	40	3.6	1372.80
2	PKC	13	1.95	390
3	Soya(SBM)	34	14.28	938.40
4	Fish	5	3.25	143.50
5	Wheat	2.5	0.425	46.75
6	Bone meal	4		
7	Salt	0.5		
8	Lysine	0.3		
9	Methionine	0.2		
10	Premix	0.5		
	Total	100	23.51	2891.45

Table 1: Coposition of the experimental super broiler starter

Materials and Methods

Location

The study was carried out at the Veterinary Farm, University of Nigeria, Nsukka. Nigeria.

Materials

Probiotic yeast (***Sacharomyces cerevisiae***) obtained from B.F.P. Doak Road, Felixstowe, United Kingdom.

Commercial enzymes (Marketed as zyme(R))

Experimental diets: Broilers (day-old chicks. Ross breed)

Methods

Experimental animals/design: Eighty day –old broiler chicks were randomly divided into 4 groups

(P1-P4) of 20 birds each. Each group was subdivided into 5 replicates of 4 birds. P1 had no probiotic in their diet and no enzymes in their water (control) (Table 2).

P2 had enzymes in water (0.02 ml/lit) but no probiotic in the diet. P3 had enzymes in their water (0.02 ml/lit) and probiotic (0.08 g/kg) in their feed. P4 had probiotic in their feed (0.8 g/kg) but no enzymes in their water. They were given feed and water ad libitum. The quantity of feed and water consumed each day were determined. The birds were weighed weekly. The study lasted for 6 weeks.

Vaccination and health management: All the birds were routinely vaccinated as recommended By Nigerian Veterinary Institute, Vom, Nigeria. They closely observed for any sign of ill health.

Haematology and Serum Biochemistry

Sample collected: Blood: At the 6th week of age, 5 birds were randomly selected from each group (one from each replicate) and 5 ml of blood was collected from each bird from the external jugular vein with sterile syringe and needle. Blood collected were put in a labeled test tube and allowed to clot. The serum harvested was used for the blood biochemical components assay following standard procedures. Another set of 5 birds were similarly selected and 3 ml. of blood collected from same site and put into a sample bottle containing EDTA for haematological determinations following standard procedures (Table 3).

Data analysis: The weekly weight gain, feed intake and water consumption were analyzed using repeat Measure Analysis of Variance (ANOVA) in SPSS window 15.0. Other parameters were analyzed with

S/N	Ingredient	%	CP (%)	ME(Kcal/kg)
1	Maize	46	4.14	1579.64
2	PKC	13	1.95	390
3	Soya (SBM)	28	11.76	756
4	Fish meal	5	3.25	143
5	Wheat	2.5	0.425	46.75
6	Bone meal	4		
7	Salt	0.5		
8	Lysine	0.3		
9	Methionine	0.2		
10	Premix	0.5		
	Total	100	21.53	2915.3

Table 2: Composition of the Experimental Broiler Starter

s/n	Ingredients	%	CP (%)	ME(kcal/kg)
1	Maize	52	4.68	1789.64
2	PKC	12	1.8	360
3	Soya (SBM)	24	10.08	648
4	Fish	5	3.25	108.75
5	Wheat offal	1.5	0.21	27.75
6	Bone meal	4		
7	Salt	0.5		
8	Lysine	0.3		
9	Methionine	0.2		
10	Premix	0.5		
	Total	100	20.02	2934.14

Table 3: Composition of the Experimental Broiler Finisher

	P1(control)	P2(enzyme only)	P2(enzyme only)	P4 (probiotic only)
WEEK 0	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
WEEK 1	0.26 ± 0.01^{ab}	0.24 ± 0.01^{a}	0.24 ± 0.01^{a}	0.29 ± 0.00^{b}
WEEK 2	0.55 ± 0.02	0.50 ± 0.02	0.50 ± 0.02	0.55 ± 0.01
WEEK 3	0.89 ± 0.04	0.91 ± 0.05	0.91 ± 0.05	0.89 ± 0.01
WEEK 4	1.16 ± 0.02^{a}	1.17 ± 0.04^{a}	1.17 ± 0.04^{a}	1.34 ± 0.01^{b}
WEEK 5	1.82 ± 0.03^{a}	1.86 ± 0.07^{ab}	1.86 ± 0.07^{ab}	2.08 ± 0.08^{b}
WEEK 6	2.31 ± 0.02^{a}	2.33 ± 0.08^{a}	2.33 ± 0.08^{a}	2.51 ± 0.05^{b}

Different superscripts ^{a,ab,b} in a row indicate significant differences between the means (p≤0.05).

Table 4: Mean Weekly Weight of Broilers Fed Diets Supplemented With Probiotic and Commercial Enzyme (Kg/Bird)

one – way ANOVA. Group means were compared using Duncan new multiple range test. Significance level was accepted at p<0.05.

Results

Results of the mean weekly weight are presented in Table 4. The live weight gain of the treatment groups were significantly different (p<0.05) at WK1, WK4, WK5 and WK6. Highest body weight gain was recorded by group P4 (Probiotic only).

The results of serum biochemical components are presented in Table 5. The results revealed an increase in AST among the treated groups. The mean levels of ALT, ALP, Total Protein, Cholesterol, Albumin, Creatinine, Albumin/Globulin ratio showed no significant difference (P>0.05).

Haematological results are shown in Table 6. There was no significant difference (P>0.05) in the Pack cell volume, Red blood cell count, Mean corpuscular hemoglobin, White blood cell count, Heterophils, Lymphocytes, Monocytes and Basophils. However, Eosinophil count was significantly (P<0.05) higher in P3 (probiotic and enzyme) and P4 (probiotic only) than P1 (control) and P2 (enzyme only) (Table 6).

Weekly water consumption and feed intake are shown in Tables 3 and 4 respectively. There were no significant differences (P>0.05) in both water and feed consumption among the experimental groups.

Discussion

The combined and individual effects of probiotic supplementation in the diet and enzyme in drinking water on broiler chicken were investigated. The study showed significant difference (P<0.05) in mean weekly weights at weeks 1, 4, 5 and 6. P4 weighed significantly (P<0.05) heavier than other groups at weeks 1, 4, 5 and 6 followed by P3. The increased weight gain observed in P4 and P3 may be due to the activities of the probiotic which elaborates digestive enzymes such as sucrase and phytase that help in nutrient digestion thereby promoting growth of these groups [16]. This is supported by the findings of Matsui et al. who reported that *S. cerevisiae* elaborates digestive enzymes which help the host enzymes to increase digestibility and improve efficiency of feed utilization and weight gain. It has also been shown that probiotics breakdown feed into smaller substances making their digestion and absorption by the host animal easier [17-19] (Tables 7 and 8).

The study revealed that there was no significant difference (p>0.05) in weight gain performance between P2 (enzyme in water only) and P1 (control). This could be due to the type of enzyme(s) contained in the preparation. The experimental diet was a high fibre diet because of the palm kernel cake which is usually included in our environment to reduce cost of poultry feed. If the commercial enzymes do not include cellulases or hemicellulases, sucrases and phytases, it may not exert significant influence on weight gain performance. Most of the commonly available commercial enzyme preparations for supplementation in feeds are known to contain mainly proteases and amylases.

The results of the experiments showed no significant difference (P>0.05) in feed and water consumption among the experimental groups. This finding is in agreement with earlier observations by Adejumo et al. [20] and Ezema [5]. Although, Craig et al. [7] observed that enzyme supplementation increased feed intake. Since the probiotic supplemented groups showed higher weight gain and there were no significant difference (p<0.05) in both feed and water intake, it suggests that the probiotic supplemented groups had higher efficiency of feed utilization and consequently superior feed conversion ratio.

The study revealed no significant difference (P>0.05) in the Pack cell volume, Red blood cell, Mean corpuscular haemoglobin, White blood cell, Heterophils, Lymphocytes, Monocytes, Basophils. These results are in disagreement with the earlier findings of Jin et al. [21] who reported that probiotic increased the haematological profile of poultry either due to its direct effects on haemopoetic organs or the indirect effects on the intestinal micro flora. However, haematological parameters are always influenced by environmental changes and nutrition.

There was a significant difference (P<0.05) in Eosinophil count between P3 (probiotic and enzyme) and P4 (probiotic only) compare to P1 (control) and P2 (enzyme only). This could be caused by the influence of the probiotic and enzyme on homural immune system [22].

The study of serum biochemistry showed significant (p<0.05) difference in AST level among the treated groups compared to the control. Charles and Margi [23] reported that AST is considered a liver enzyme. However, AST is also found in significant amounts in many other tissues, including cardiac muscles, skeletal muscle, kidneys and pancreas. The increase in AST may be a reaction of these organs to the presence of these supplements.

Group P3 had lower mean weight gain than P4 and this suggests that the combination of the probiotics and the enzyme was not synergistic and therefore not recommended.

Conclusion and Recommendation

Probiotic supplementation significantly increased weight gain but enzymes supplementation did not significantly increase weight gain.

Based on the findings of this study, combination of probiotic and enzyme did not do better than probiotics alone in weight gain

Parameters	**P1(control)**	**P2(enzyme only)**	**P3(enzyme + probiotic)**	**P4(probiotic only)**
ALT (IU/L)	45.07 ± 0.15	45.17 ± 0.07	45.16 ± 0.09	45.10 ± 0.16
AST (IU/L)	57.00 ± 1.73[a]	60.73 ± 0.07[b]	60.77 ± 0.08[b]	60.52 ± 0.22[b]
ALP (IU/L)	240.54 ± 14.95	253.75 ± 7.04	246.88 ± 17.53	260.94 ± 12.80
Total Cholest. (mg/dl)	**137.63 ± 10.65**	**116.40 ± 1058**	**127.06 ± 18.39**	**95.24 ± 18.38**
Total Prot. (g/dl)	2.45 ± 0.11	2.77 ± 0.13	2.49 ± 0.22	2.51 ± 0.13
Albumin (g/dl)	1.43 ± 0.13	1.33 ± 0.10	1.29 ± 0.06	1.40 ± 0.12
Globulin (g/dl)	1.02 ± 0.04	1.44 ± 0.17	1.20 ± 0.25	1.12 ± 0.24
Albumin/Globulin Ratio	1.42 ± 0.17	0.97 ± 0.19	1.19 ± 0.25	1.47 ± 0.51
Creatinine (m/dl)	0.56 ± 0.11	0.67 ± 0.00	0.56 ± 0.11	0.56 ± 0.11

Different superscripts a,b in a row indicate significant difference between the groups (P<0.05).

Table 5: Serum Biochiacl profile (Means ± standard error) of broilers fed diets supplemented with probiotic and enzyme.

PARAMETERS	**P1(control)**	**P2(enzyme only)**	**P3(enzyme + probiotic)**	**P4(probiotic only)**
pcv (%)	29.50 ± 0.50	29.17 ± 0.44	29.33 ± 0.60	29.17 ± 0.60
RBC(10^6/µL)	2.51 ± 0.09	2.45 ± 0.03	2.55 ± 0.07	2.32 ± 0.04
HBC(g/dl)	9.31 ± 0.30	8.45 ± 0.91	8.79 ± 0.30	8.45 ± 0.34
MCV(fl)	117.75 ± 5.48	118.81 ± 3.37	115.17 ± 5.43	125.84 ± 4.15
MCH(pg)	37.07 ± 0.70	34.31 ± 3.35	34.43 ± 0.36	36.41 ± 4.15
MCHC(mg/dl)	31.58 ± 1.16	29.07 ± 3.54	30.03 ± 1.48	28.94 ± 0.63
WBC(10^3/µl)	13.22 ± 0.90	21.98 ± 6.16	20.78 ± 1.17	18.45 ± 3.32
HET(10^3/µl)	2.97 ± 0.39	6.76 ± 2.26	6.67 ± 1.28	6.17 ± 1.68
LYM(10^3/µl)	9.89 ± 0.54	14.31 ± 3.64	13.56 ± 0.27	11.43 ± 1.89
MON(10^3/µl)	0.08 ± 0.08	0.16 ± 0.10	0.27 ± 0.06	0.24 ± 0.06
EOS(10^3/µl)	0.21 ± 0.03[a]	0.54 ± 0.10[b]	0.42 ± 0.02[ab]	0.37 ± 0.07[ab]
BAS(10^3/µl)	0.05 ± 0.05	0.17 ± 0.10	0.14 ± 0.07	0.16 ± 0.10

Different superscripts a,b,ab in a row indicate significant difference between the groups (P<0.05).

Table 6: The haematological profile (Means ± standard error) of broilers Fed diets supplemented with probiotic and enzymewith probiotic and enzyme.

WEEK	**P1(control)**	**P2(enzyme only)**	**P3(enzyme + probiotic)**	**P4(probiotic only)**
1.	1.63 ± 0.07	1.88 ± 0.21	1.96 ± 0.25	1.95 ± 0.22
2.	3.08 ± 0.28	2.97 ± 0.21	3.20 ± 0.40	3.50 ± 0.27
3.	4.41 ± 0.41	4.65 ± 0.39	4.94 ± 0.21	4.84 ± 0.13
4.	6.79 ± 0.19	6.96 ± 0.11	6.98 ± 0.21	7.04 ± 0.22
5.	8.65 ± 0.28	8.36 ± 0.17	8.41 ± 0.29	8.83 ± 0.29
6.	9.13 ± 0.64	8.54 ± 0.74	8.45 ± 0.61	9.78 ± 0.97

Table 7: Mean weekly water consumption of broilers fed diets supplemented with probiotic and commercial enzyme (lit./bird).

WEEK	**P1**(CONTROL)	**P2**(enzyme only)	**P3**(enzyme + probiotic)	**P4**(probiotic only)
1.	0.79 ± 0.07	0.82 ± 0.11	1.08 ± 0.30	1.01 ± 0.25
2.	1.57 ± 0.10	1.49 ± 0.12	1.89 ± 0.15	2.10 ± 0.08
3.	2.15 ± 0.13	2.09 ± 0.14	2.46 ± 0.09	2.50 ± 0.04
4.	2.68 ± 0.14	2.81 ± 0.10	2.81 ± 0.11	2.65 ± 0.20
5.	3.64 ± 0.15	3.76 ± 0.14	3.63 ± 0.11	3.91 ± 0.24
6.	3.94 ± 0.42	3.46 ± 0.51	3.93 ± 0.43	4.30 ± 0.51

Table 8: Mean weekly feed intake of broilers fed diets supplemented with probiotic and commercial enzyme (kg/bird)

performance. Hence, probiotic supplementation alone is recommended for improved broiler production in this environment and using this type of diet.

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