BIOCHEMISTRY, DIFFERENTIAL COUNT AND LIVER FUNCTION PROFILE OF BROILER CHICKENS SERVED DIETARY INCLUSION OF PROBIOTICS (*SACCHAROMYCES CEREVISIAE*) AND ENZYME (BIO-ENZYME)

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ABSTRACTS
This study was conducted to evaluate the biochemistry, differential count and liver function profiles of broiler chickens served dietary inclusion of probiotics (*Saccharomyces cereviasae*) and enzyme (Bio-enzyme). One hundred and twenty day-old broiler chicks of Agrited strain were used for the study. The birds were randomly allotted to four dietary treatments. Each treatment contained 30 birds replicated three times to contain 10 birds per replicate in a completely randomized design experiment. The four dietary treatments were T1 Control (no probiotics and enzyme), T2 (had both probiotics and enzyme), T3 (probiotics only) and T4 (Enzyme only). The probiotics and enzyme was included at 0.8% and 0.02%, respectively. The birds were given feed and water *ad-libitum*. The experiment lasted eight weeks. At the end of the experimental period, three birds were randomly chosen from each replicate for biochemistry, differential count and liver function profiling. The results showed that inclusion of Saccharomyces cerevisiae and bio-enzyme had significant effect ($p<0.05$) on the biochemistry, differential count and liver function profile. The dietary inclusion of probiotics and enzyme reduced total cholesterol and low density lipoprotein, gut and intestinal microoganisms and favoured the functionality of the liver of the broiler chickens. It was therefore concluded that probiotics and bioenzyme should be included in broiler diets for immunity improvement and reduction of cholesterol levels.
Key words: Broilers, probiotics, enzyme, biochemistry, differential count and liver function

INTRODUCTION
Antibiotics as a growth promoter have been widely used extensively in poultry feed for more than 50 years (Haque*et al.*, 2017), playing a crucial role in promoting chicken growth and lowering enteric diseases. However, the deleterious effect of this product on the health of the consumers cannot be overemphasized. In Europe and South Korea, growth promoting antibiotics have been banned since 2006 and 2012 respectively, and these bans are expected to affect the rest of the world (Aude*et al*., 2015). Hence, tremendous research has been geared towards finding alternatives to antibiotics that would maintain low mortality rate, good level of animal yield while preserving environment and consumer health. Several alternative strategies have been proposed with some success that mimics the function of antibiotics as growth promoters and these include the use of probiotics, prebiotics, organic acids, and exogenous enzyme, among others (Yadav and Jha, 2019).
Probiotics are living microorganisms that confer health benefits to the host when administered in adequate amounts. The most important advantage of probiotics is that they neither have any residues in animal products nor exerts any antibiotic resistance on consumption while having good impact on the poultry performance. The use of probiotics in poultry production is a recent practice which has progressed from the transplantation of feces from healthy adult hens into neonatal chicks to a more mature technology focused on developing probiotics formulation to address specific, wide-ranging issues relevant to today's modern poultry industry (Wolfenden and Hargis, 2020). In broiler nutrition, probiotic species belonging to Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida and Saccharomyces have a beneficial effect on broiler performance, modulation of intestinal flora, intestinal histological changes, immunomodulation, in addition, have an impact on specific haemato-biochemical parameters and improving microbiological meat quality of broilers Ashayerizadeh*et al*., 2009).
The role of exogenous enzymes is to fulfill the absence of endogenous enzymes, to counter the anti-nutritional factors present in conventional and unconventional poultry diet. Probiotics and exogenous enzymes can also enhance broiler productivity and likely immunity too, that may represent an alternative to the use of sub-therapeutic doses of antibiotics, meeting the growing public concerns on the contribution of this practice to the increasing antibiotic resistance (Yadav and Jha, 2019). As information on the effect of a mixture of probiotic microorganisms and enzymes on the immunity of broilers are very limited, this study intended to determine the effect of different dietary levels of a

probiotic (*Saccharomyces cereviasae*) and enzyme (bio-enzyme) inclusion to broiler chickens on their immune response, lipid profile and liver function profile.

MATERIALS AND METHODS

Study location

The experiment was conducted at the Poultry Unit, Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka, Enugu State, Nigeria. Nsukka is in Enugu state, Nigeria

Experimental design, animals and management

The study was conducted to evaluate the biochemistry, differential count and liver function profiles of broiler birds served dietary inclusion of probiotics (*Saccharomyces cereviasae*) and enzyme (Bio-enzyme). One hundred and twenty day-old broiler chicks of Agrited strain were used for the study. The birds were randomly divided into four groups and assigned to the four dietary treatments. Each treatment contained 30 birds replicated three times to contain 10 birds per replicate in a completely randomized design. The four dietary treatments were T1 Control (no probiotics and enzyme), T2 (both probiotics and enzyme), T3 (probiotics only) and T4 (Enzyme only).The probiotics and enzyme was included at 0.8% and 0.02%, respectively. The birds were given feed and water *ad-libitum* and the quantity of feed consumed each day were determined through weigh back techniques. The birds were weighed weekly throughout the experimental period and the experiment lasted for eight weeks. Vaccination and medications were provided as when due and other routine management practices were provided accordingly. At the end of the experimental period, three birds were randomly chosen from each replicate for biochemistry, differential count and liver function profiling. Blood samples were collected into EDTA containers with the aid of sterile syringes and needles and taken to the laboratory for analysis.Proximate analysis of feeds was done according to AOAC (1990). Data collected were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, 2013) version 19.00. Significant different means were separated using Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The effect of probiotics (*Saccharomyces cereviasae*) and enzyme (bio-enzyme) inclusion on the lipid profile of broiler birds is presented in Table 2. The serum total cholesterol, LDL, HDL and triglyceride concentrations were significantly ($P<0.05$) reduced in broiler chickens fed treatments that were supplemented with *Saccharomyces cereviasae*and bio-enzymes when compared to the control treatment. The value of Total.Chol (3.99g/l), TG (1.61g/l) and HDL (0.79g/l) were lowest in the treatment group with only probiotics while LDL value (2.18g/l) was lowest in the treatment group with mixture of *Saccharomyces cereviasae*and bio-enzymes dietary inclusion. The value of total cholesterol and TG obtained in this work was in line with that of Haque*et al*. (2017) who reported highest serum total cholesterol, and TG level in the control group compared to all other groups supplemented with either antibiotics or probiotics. Similarly, the result concurred with the result of Shokryazdan*et al*. (2017) who observed that the supplementation of the three L. salivarius strains to broilers significantly reduced their serum LDL, but did not affect HDL levels at 21 and 42 days of age. This result is also in agreement with Saleh*et al*.(2012) who reported that the inclusion of Aspergillusawamori and Saccharomyces cerevisiae in chickens feed reduced blood saturated fatty acids and increased the polyunsaturated.

The effect of probiotics (*Saccharomyces cereviasae*) and enzyme (bio-enzyme) inclusion on the differential count of broiler birds is presented in Table 3.Neutrophil and lymphocyte were lower in treatment groups with dietary inclusion of probiotics. However, the neutrophil and lymphocyte counts were significantly ($p<0.05$) different in the control treatment groups. Monocyte and eosinophil count were significantly ($p<0.05$) different in the treatment group with a mixture of probiotics and enzyme with the value 2.33% and 3.67% for monocyte and eosinophil respectively. There were no significant differences in the basophil count across the treatment groups. This result perfectly agrees with the work of Mohamed *et al*. (2019) who reported a significant reduction in lymphocyte percentage by treatment groups with dietary inclusion of probiotics. This implies that probioticsimproves the

immune system of broiler chicken. This improvement is achieved by reducing intestinal pH, intestinal bacteria composition and digestive activity. Probiotic bacteria produce molecules with antimicrobial activities such as bacteriocins which inhibits toxins' production and pathogens' adhesion. On the other hand, probiotics stimulate the immune response and increase resistance to colonization of bacteria (Hassanein and Soliman, 2010).
The effect of probiotics (*Saccharomyces cereviasae*)and enzyme (bio-enzyme) inclusion on the liver function profile of broiler birds is presented in Table 4.AST, ALT and ALP activities were significantly ($p<0.05$) different in treatment groups with bio-enzyme compared to the control groups and the treatment group with only probiotics. The activities of Tot.Bili and D.Bili were lower in treatment groups with probiotics or enzyme. The decrease in AST, ALT and ALP value in the probiotics treated groups perfectly aligns with the work of Haque et al., 2017 who measured highest value in the control treatmentsand least value in probiotic supplemented group. These findings concur with the finding that the supplementation of probiotics lowers AST and ALT level. Mechanisms of action of probiotics include stimulation of endogenous enzymes, reduction of metabolic reactions that produce toxic substances, and production of vitamins or antimicrobial substances (Hassanein and Soliman, 2010).

CONCLUSION

Dietary inclusion of *Saccharomyces cerevisiae*significantly lowered blood cholesterol and glucose levels in broiler which are beneficial parameters to enhance broiler production. Immune response, lipid profile and liver function profile of broiler chicken were improved by the treatment with probiotics inclusion (*Saccharomyces cerevisiae*). In this regard *Saccharomyces cerevisiae*should be included in the diet of broiler chicken as alternative to the use of antibiotics growth promoters.

Table 1: The percentage composition of the experimental diets of the broiler chickens

Parameters	**Treatments**			
	T1 (Control)	**T2 (Probiotics and Enzyme)**	**T3 (Enzyme only)**	**T4 (Probiotics only)**
Maize	53.00	53.00	53.00	53.00
Wheat offal	10.00	10.00	10.00	11.00
Palm kernel cake	5.50	5.50	5.50	5.50
Groundnut cake	15.00	15.00	15.00	15.00
Soya bean meal	10.00	10.00	10.00	10.00
Fish meal	1.50	1.50	1.50	1.50
Bone meal	4.00	4.00	4.00	4.00
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Vit-Premix	0.25	0.25	0.25	0.25
Probiotics	-	0.80	-	0.80
Enzyme	-	0.02	0.02	-
Total	100.00	100.00	100.00	100.00
Calculated composition				
CP%	20.12	20.01	20.11	20.04
Energy (MJME/kg)	3.68	3.81	3.75	3.71

Table 2: Effect of Probiotics and Enzyme inclusion on the lipid profile of Broiler birds

Parameters	T1 (Control)	T2 (Prob. &Enz.)	T3 (Enzyme only)	T4 (Prob. Only)
Tot. Chol. (g/l)	5.07±0.07[a]	4.22±0.02[c]	4.40±0.06[b]	4.49±0.01[d]
Trig. (g/l)	1.90±0.00[a]	1.79±0.02[b]	1.89±0.00[a]	1.61±0.02[c]
HDL (g/l)	1.50±0.03[a]	1.20±0.00[c]	1.40±0.01[b]	0.79±0.05[d]
LDL (g/l)	3.83±0.60[a]	2.18±0.12[c]	2.78±0.10[b]	2.92±0.02[b]

Tot. Chol = Total Cholesterol, Trig. = Triglyceride, HDL = High density lipoprotein, LDL = Low density lipoprotein, Prob. = Probiotic, Enz. = Enzyme.

Table 3: Effect of Probiotics and Enzyme inclusion on the Differential count of Broiler birds

Parameters	T1 (Control)	T2 (Prob. &Enz.)	T3 (Enzyme only)	T4 (Prob. Only)
Neut. (%)	68.33±0.33[a]	56.00±2.00[b]	70.33±1.53[a]	52.00±0.00[c]
Lymph (%)	45.67±0.33[a]	38.00±1.16[b]	26.00±1.16[d]	30.67±0.33[c]
Eosi (%)	2.33±0.33[b]	3.67±0.33[a]	2.00±0.00[b]	2.33±0.33[b]
Mono (%)	0.00±0.00[b]	2.33±0.03[a]	0.00±0.00[b]	0.00±0.00[b]
Baso (%)	0.00±0.00	0.00±0.00	1.00±0.00	0.00±0.00

Neut. = Neutrophil, Lymph. = Lymphocyte, Eosi. Eosinophil, Mono. = Monophil, Baso. = Basophil, Prob. = Probiotic, Enz. = Enzyme.

Table 4: Effect of Probiotics and Enzyme inclusion on the Liver function profile of Broiler birds

Parameters	T1 (Control)	T2 (Prob. &Enz.)	T3 (Enzyme only)	T4 (Prob. Only)
AST (µl)	124.33±1.45[c]	150.67±1.45[a]	132.00±1.16[b]	114.67±0.33[d]
ALT (µl)	27.17±0.33[c]	29.03±0.03[b]	21.00±0.29[d]	280.67±0.67[a]
ALP (µl)	10.33±0.33[d]	15.67±0.03[b]	17.00±0.00[a]	12.00±0.58[c]
Tot. Bili (mg/dl)	1.47±0.03[a]	1.43±0.03[a]	1.30±0.06[b]	1.30±0.00[b]
D. Bili (mg/dl)	0.30±0.03[d]	0.41±0.01[c]	0.87±0.03[a]	0.70±0.03[b]

AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = *Alkaline phosphatase,*Prob. = Probiotic, Enz. = Enzyme.

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