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SERUM BIOCHEMISTRY AND HAEMATOLOGICAL ASPECTS OF FEEDING BROILERS WITH FERMENTED RICE WASTE, PALM KERNEL CAKE AND SPENT GRAIN

Ubi, J.[1*]; Eneh, C.A.[2]; Onyimonyi, A. E.[3]; Ugwu, S.O.C.[3]
[1] Biotechnology Centre, Enugu
[2] Department of Agricultural Economics, University of Nigeria, Nsukka
[3] Department of Animal science, University of Nigeria, Nsukka
[*] Corresponding Author, ubijacob4@gmail.com

Abstract
The effect of feeding broilers rice waste, palm kernel cake, and spent grain fermented with liquid spent brewer's yeast on haematology and serum biochemistry were studied for eight weeks. During the starting phase, 108 day-old Agritted broiler chickens were brooded and fed commercial ration for 2 weeks. They were weighed at 3 weeks old and were assigned to 4 different treatments (each with 27 birds) in a completely randomized design. Each group was randomly assigned to one of the 4 starter diets (0kg fermented diet for control and 21.07kg of rice waste, palm kernel cake and spent grain fermented with LSBY; 21.56-23.20% CP) and 4 finisher diets (0kg fermented diet for control and 24.15kg of rice waste, palm kernel cake and spent grain fermented with LSBY; 19.07-19.92% CP). Each treatment was replicated thrice with 9 birds each; feed and water were provided ad-libitum. Among the data collected for haematological study, significant (P<0.05) differences were obtained for RBC, Hb and PCV; whereas for serum biochemistry, significant (P<0.05) variations were obtained for ALT, AST, ALP and urea.

Keywords: Liquid spent brewer's yeast; Broiler; Blood parameters

Introduction

The livestock business in Nigeria is the primary source of animal protein. Poultry production outnumbers all other forms of livestock in Nigeria, and it is not unexpected that it is found all around the country (Adeyemo and Onikoyi, 2012). According to FDLPC (2004), Nigeria produced 2 billion eggs and 12,000 tons of chicken meat, with 1.3 kg of poultry meat consumed per person (Okaiyeto and Adamu, 2004). Poultry, which is Nigeria's second-largest source of protein after ruminants, accounts for over a quarter of the country's meat production. Animal protein is required in human nutrition to address the problem of kwashiorkor, a malnutrition-related condition (Oladeebo *et al*., 2007). However, for an adult weighing 60 kg, the Food and Agricultural Organization recommends 36 grams of animal protein per day (FAO, 2006). Ani *et al*. (2013) opined that the demand for protein of animal origin in Nigeria is larger than the supply as a direct and indirect result of the country's growing population.

Rapid advancement in growth-promoting feed supplements or additives for animal nutrition has resulted in the growing demand for safe and high-quality chicken products (Ruiz *et al*., 2015). Thus, the fortification of some feed ingredients (rice waste, palm kernel cake and spent grain) with liquid spent brewer's yeast (LSBY) as a natural product could be an avenue for the improvement of feed nutritive value leading to a better health and improvement of the animal (broiler). LSBY (*Saccharomyces cerevisiae*) is high in biologically essential proteins, B-complex vitamins, trace minerals, and a variety of unique "plus factors." The availability of phosphorus has also been established as a positive factor (Brake, 1991; Moore *et al.*, 1994), minimizing disease infections (Line *et al.*, 1997), feed efficiency increment (Onifade and Babatunde, 1996) and nutrient uptake (Pagan, 1990). Nevertheless, there are indeed mixed studies on the benefits of using yeast in poultry diets. According to Raven and Walker (1980), about 45% of *S. cerevisiae* is protein, 2.7% is crude fibre and 1% is lipid. It has an excellent amino acid profile, although it suffers from a lack of sulphur-containing amino acids like methionine and cystine, as well as a high lysine concentration (Huige, 2006).

Two out of four novel yeast applications in animal production have developed in recent years, both of which are beyond the traditional use. These models are: (i) the involvement of certain yeasts in modifying the gut microbiota of animals and stimulating the immune system; (ii) when given to feed, *Saccharomyces cerevisiae* can prevent aflatoxicosis in broiler chickens (Gheisari and Kholeghipour, 2006). Onifade *et al*. (1999) found that rabbits fed a 3.0 g/kg supplemental diet of cultured *S. cerevisiae* had better haematological parameters than rabbits provided unsupplemented feed. Furthermore, other yeast like *Bifidobacterium infantis* and *Lactobacillus plantarum* in rat's diet led to a reduction in the alanine aminotransferase (ALT) activity according to Osman et al. (2007); whereas inclusion of *S. cerevisiae* statistically increased the activities of serum alkaline phosphatase (ALP) and ALT (Mannaa *et al*., 2005). Hayat *et al*. (1993) reported that effect of *Saccharomyces* dried yeast in birds may be regulated by the genetic makeup of the animal.

The aim of the study was to evaluate the serum biochemical and haematological aspects of broiler birds on rations composed of rice waste, palm kernel cake and spent grain fermented with liquid spent brewer's yeast (*Saccharomyces cerevisiae*).

Materials and Methods

Location and Duration of the Study

The experiment was conducted at the University of Nigeria, Nsukka's Poultry Unit of the Department of Animal Science Teaching and Research Farm. This research lasted for a period eight (8) weeks.

Experimental Birds and Management

The guidelines on research ethics involving animal subjects published by the University of Nigeria, Nsukka (2013) were used to conduct this study. The investigation employed 108 day-old *Agritted* broiler birds obtained from an Agritted supplier in Nsukka, Enugu State. Chicks were brooded for two weeks before being randomly assigned to four treatments of 27 birds each on the third week of the trial in a completely randomized design (CRD). Each group was replicated three times, with nine birds in each replicate. The birds had unrestricted access to water and food during the study.

Procurement and Processing of Experimental Materials

The Nigerian Brewery Industry at 9th Mile, Enugu State provided the LSBY. Rice waste, spent grain, and palm kernel cake were also obtained from the Nsukka market in Enugu State. The LSBY was mixed with rice trash, palm kernel cake, and spent grain in a 1:3 ratio and ensiled for 2 weeks in polythene bags; that is, 1 litre of LSBY in 3kg of rice waste, palm kernel cake, and spent grain. As a result, for the starter diet, 7.5 litres of LSBY were combined with 21.07kg of rice waste, palm kernel cake and spent grain, while for the finisher diet, 8.5 litres of LSBY were combined with 24.15kg of the test ingredients. The end products were all gathered separately for drying after 2 weeks of fermentation in ensilage, which helped to minimize moisture content. Broiler starter and finisher meals included the fermented diets.

Experimental Diets

Table 1 and 2 presents the percentage proportion of the compounded rations at different phases of development (starter and finisher mashes) and the proximate composition of the diets.

Table 1: Percentage proportion of the experimental diets

	Starter				Finisher			
Starter feedstuff (%)	**Cont.**	**T_1**	**T_2**	**T_3**	**Cont.**	**T_1**	**T_2**	**T_3**
Maize	42.14	21.07	21.07	21.07	48.29	24.14	24.14	24.14
Wheat offal	17.89	18.05	21.86	30.86	20.70	20.70	24.71	35.40
Soybean meal	31.97	31.81	30.00	21.00	23.01	23.01	21.00	11.31
Fish meal	4.00	4.00	2.00	2.00	4.00	4.00	2.00	1.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral premix®	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
FRW	0.00	21.07	0.00	0.00	0.00	24.15	0.00	0.00
FPKC	0.00	0.00	21.07	0.00	0.00	0.00	24.15	0.00
FSG	0.00	0.00	0.00	21.07	0.00	0.00	0.00	24.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition								
Crude protein (%)	23.20	21.69	22.14	21.56	19.31	19.92	19.48	19.07
Crude fiber (%)	11.00	11.00	6.50	9.50	5.00	9.50	6.50	9.50
Fat (%)	1.00	1.00	2.50	0.50	1.50	3.50	2.50	0.50
Energy (MJ/kg ME)	2324.15	2378.16	2767.43	2479.53	2296.85	2494.04	2767.43	2479.53

T_1: Rice waste; T_2: Palm kernel cake; T_3: Spent grain; Cont.: Control; FRW: fermented rice waste; FPKC: fermented palm kernel cake; FSG: fermented spent grain; Vit-min-premix used in this research: vit A: 106 (IU); vit D_2: 500,000 (IU): vit E: 2000 (IU); vit B_1: 1000 (mg); vit B_2: 1500 (mg); vit B_6: 1000 (mg); vit B_{12}: 10,000 (mg); Ca Pantothenate: 3000 (mg); biotin: 5000 (mg); niacin-amide: 10,000 (mg); folic acid: 100 (mg); Na bicarbonate: 250,000 (mg); NaCl: 10,000 (mg); KCl: 20,000 (mg).

Table 2: Proximate composition of the ensiled rice waste, spent grain and palm kernel cake using liquid spent brewer's yeast (LSBY)

Samples (%)	FRW, ratio 1:3	FPKC, ratio 1:3	FSG, ratio 1:3
Crude protein	9.19	15.76	30.65
Ash	17.00	14.00	7.50
Moisture	12.00	23.00	20.00
Ether extract	2.06	10.00	3.50
Crude fiber	17.00	20.50	19.00
N-Free extract	42.75	16.74	19.35

FRW: Fermented rice waste; FPKC: Fermented palm kernel cake; FSG: Fermented spent grain

Data Collection for Haematology and Serum Biochemical Indices

At the end of the experiment, blood samples of approximately 2.5ml each were taken from the wing veins of 3 birds from each replicates. For the determination of haematological indices, EDTA bottles were used for the collection whereas the other was obtained in plain tubes excluding EDTA. The micro haematocrit reader was used to calculate the packed cell volume (Baker and Silverton 1985). Baker and Silverton (1985) methods for calculating red blood cell count and haemoglobin were used. An automated hematology analyzer was used to calculate the number of white blood cells (WBCs). Centrifugation at 6000 rpm for 1.30 minutes isolated the serum, which was then analyzed. ALT, ALP and AST were measured using commercial kits [ALP (AP307), AST (AS101) and ALT (AL100) – Randox, United Kingdom] and a spectrophotometer. AST and ALT were measured at 546 nm while ALP measured at 405 nm. Haematological indices obtained are: red blood cells, packed cell volume, white blood cells, haemoglobin concentration, basophils, neutrophils, lymphocytes, monocytes and eosinophil. Whereas, liver function parameters measured included: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate. For kidney function, urea, creatine and bilirubin were the parameters collected.

Experimental Design and Data Analysis

A Completely Randomized Design (CRD) was used to conduct this research, with a statistical model as follows:

$$X_{ij} = \mu + T_i + C_{ij}$$

Where;

Xij = Overall observation
μ = Population mean
Ti = Treatment effect
Cij = Experimental error

Using the statistical package (SPSS, version 20), data were subjected to analysis of variance (ANOVA), and statistical different means were separated using Duncan's New Multiple Range Test procedures (Duncan, 1955).

Results and Discussion

Results on the haematology and serum biochemical indices of broilers fed rice waste, palm kernel cake and spent grain fermented with liquid spent brewer's yeast (LSBY) are presented in Table 3 and 4.

Table 3: Hematological indices of broiler birds fed rice waste, palm kernel cake and spent grain fermented with liquid spent brewer's yeast (LSBY)

Parameters	Control	T_1	T_2	T_3	P-Value
RBC ($x10^6 mm^3$)	$9.92±0.25^a$	$7.42±0.53^b$	$7.63±0.05^b$	$7.70±0.27^b$	0.002*
Hb (g/dL)	$9.33±0.20^a$	$8.83±0.26^{ab}$	$8.27±0.06^b$	$8.10±0.40^b$	0.038*
PCV (%)	$40.00±1.15^a$	$33.67±0.88^b$	$31.00±0.57^{bc}$	$30.00±1.15^c$	0.000*
WBC ($x10^{12} mm^3$)	91.33±2.67	87.00±2.08	81.00±3.78	85.00±2.08	0.138^{NS}
Neutrophils (%)	22.67±3.71	20.00±2.88	18.00±1.15	17.00±1.52	0.453^{NS}
Lymphocytes (%)	75.33±3.71	76.67±3.52	79.33±0.66	79.67±1.45	0.626^{NS}
Monocytes (%)	1.33±0.33	2.67±0.66	1.00±0.57	1.33±0.88	0.336^{NS}
Eosonophils (%)	0.33±0.33	0.67±0.33	1.00±0.57	1.33±0.33	0.400^{NS}
Basophils (%)	0.33±0.33	0.00±0.00	0.67±0.33	0.67±0.33	0.363^{NS}

^{abc}: Means with different superscripts on the same row vary statistically (*$P \leq 0.05$); T1: Fermented rice waste; T_2: Fermented palm kernel cake; T_3: Fermented spent gain; Hb: Haemoglobin Concentration; PCV: Packed Cell Volume; RBC: Red Blood Cell; WBC: White Blood Cell; NS: Non-significant difference at 1% and 5% probability level.

Table 3 has shown that, there exist statistical ($P<0.05$) variations on RBC, PCV and Hb. However, non-significant ($P>0.05$) differences were recorded on WBC, neutrophils, lymphocytes, monocytes, eosinophil and basophils. RBC for the control group showed the highest statistically ($P<0.05$) value which is different from other groups which are statistically similar. Values for Hb showed similar trend to RBC except in T_1 which is significantly similar to the control, T_2 and T_3. For PCV, control group showed the highest significant ($P<0.05$) value which is different from all other treatments. T_1 is similar to T_2 which is also similar to T_3 with the lowest PCV value.

The range of values obtained in this research are similar to those reported by Bounous and Stedman (2000) and Al-Nedawi (2018). The significant RBC of broilers in this research ranged from 7.42-9.92 $x10^6mm^3$ which is higher than 1.68-2.38 $x10^6\mu l$ reported by Lawrence-Azua *et al*. (2018). The haemoglobin and PCV obtained in this research (8.10-9.33g/dL and 30-40%, respectively) was in agreement to the published values of 9.63-11.40g/dL by Chollom *et al*. (2017) for Hb and 28-40% for PCV by Swenson (2004). An increment in these metabolites in control group as against T_1, T_2 and T_3 has shown that LSBY is capable of maintaining the health of the animal. For example, red blood cells contains haemoglobin and transport oxygen from the lungs to the periphery and as well carbon dioxide to the lungs from the periphery (Klinken, 2003); this states the important of these metabolites in maintaining the health status of the birds. Thus, from the results, fermentation with *Saccharomyces cerevisiae* did not affect the birds negatively; rather it maintained their immunity via nutritional effects. This ideology has been supported by Shanmugasundaram *et al*. (2013) when they reported that yeast product is capable of enhancing immune system of broiler birds. Panda *et al*. (2000) has also stated that yeast (*Saccharomyces cerevisiae*) has the capability of boosting immune level leading to an improved protection against disease. In a trial, yeast (*Saccharomyces cerevisiae*) in broiler chickens suppressed pathogenic bacteria, pathogenic toxin detoxification, enhancement of immune responses and nutrient availability improvement (Ha *et al*., 2006; Ivkovic *et al*., 2012).

Table 4: Serum biochemistry of broiler birds fed rice waste, palm kernel cake and spent grain fermented with liquid spent brewer's yeast (LSBY)

Parameters	Control	T_1	T_2	T_3	P. Value
ALT (Iµ/L)	56.00 ± 1.15^a	42.00 ± 1.05^b	39.67 ± 0.88^{bc}	37.67 ± 0.88^c	0.000*
AST (Iµ/L)	64.00 ± 2.31^a	53.67 ± 1.45^b	53.00 ± 2.64^b	53.67 ± 1.86^b	0.057*
ALP (Iµ/L)	34.67 ± 0.67^a	28.67 ± 3.18^{ab}	25.33 ± 1.76^b	23.67 ± 0.88^b	0.055*
Urea (mg/dL)	41.00 ± 0.57^a	31.33 ± 1.76^b	34.33 ± 2.60^b	30.67 ± 0.66^b	0.008*
Creatinine (mg/dL)	2.50±0.07	2.47±0.17	1.74±0.32	2.11±0.21	0.113^{NS}
Bilirubin (mg/dL)	5.69±0.04	4.83±0.43	5.12±0.50	4.64±0.49	0.362^{NS}

[abc]: Means with different superscripts on the same row vary statistically (*$P \leq 0.05$); T_1: Fermented rice waste; T_2: Fermented palm kernel cake; T_3: Fermented spent grain; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; NS: Non-significant difference at 1% and 5% probability level.

Results on serum biochemistry (Table 4) showed statistical ($P<0.05$) differences for ALT, AST, ALP and Urea. However, no significant variations ($P>0.05$) were obtained for Creatinine and Bilirubin. ALT and AST values of birds on the control group were highest among the treatment groups and differed significantly ($P<0.05$) from the values of T_1, T_2 and T_3, respectively. The ALP values of birds on the control were found to be statistically similar with the records of birds on T_1. Nevertheless, birds on T_1, T_2 and T_3 had statistically comparable ALP values. More so, values for urea on the control group was the highest among the treatment groups and differed ($P<0.05$) significantly from T_2, T_1 and T_3, respectively.

Significant values obtained for AST and urea in this study (53.00-64.00Iµ/L and 30.67-41.00mg/dL) are similar to the reported values of 52.25-70.00µ/L and 36.90-53.10 mg/dL by Mahmud *et al*. (2016). Our reported values for ALT (37.67-56.00Iµ/L) are lower than values of 75.00-86.40Iµ/L reported by Aluwong *et al*. (2012). Nevertheless, values of 64.75-160.50µ/L reported by Adedokun *et al*. (2017) for ALP is also higher than the reported value of 23.67-34.67 Iµ/L in this study. From this results, it could be deduced that fermentation with LSBY improved the functioning mechanism of the liver and kidney as against the control group. Elevated concentration of these metabolites in the blood shows inflammation or damage of cells that produces these enzymes (Noordam *et al*., 2017). Specifically, AST activity is found in inflammatory and degenerative liver lesions. Low ALT and AST levels when compared to the control birds, meant there was no evidence of liver disorder and this in agreement with the report of Gao *et al*. (2008). Gong *et al*. (2019) reported that dietary yeast hydrolysate which is a yeast product like the LSBY elevated antioxidative status of the body system. They also reported an up regulation of hepcidin within the liver.

Conclusion

In conclusion, fermentation of rice waste, palm kernel cake and spent grain with liquid spent brewer's yeast (LSBY) had no deleterious effect on hematological and serum biochemical parameters.

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