Effect of Graded Levels of Spent Brewers' Yeast (*Saccharomyces cerevisiae*) on Blood Parameters of Broiler Chickens

Chollom Patricia Fremu[1]*, Agbo Ediga Bede[2], Doma Umaru Dass[2], Ocheme Julius Okojokwu[1], Amos Gana Yisa[3], Fidelis Nkasiobi Ikeji[3]

[1]Department of Microbiology, Faculty of Natural Sciences, University of Jos, Jos, Nigeria.
[2]Department of Biological Sciences, Faculty of Science, Abubakar Tafawa Balewa University, Bauchi, Nigeria.
[3]Federal College of Animal Health and Production Technology, National Veterinary Research Institute, Vom, Plateau State, Nigeria.
*Corresponding Author: ciachollom@yahoo.com

Abstract: Background: The rising prices of livestock feeds and the scarcity of conventional proteins and energy concentrates for the formulation of feeds have forced the animal scientists to search for alternative, cheaper and readily available protein and energy sources. **Methods:** Five experimental diets containing 0, 5, 10, 15 and 20% spent brewers' yeast (*Saccharomyces cerevisiae*) were used to feed broiler chickens for 50 days. The experiments were in a completely randomized design with five treatments each replicated four times with 40 birds per treatment and 10 birds per replicate. **Results:** Packed cell volume (PCV) of birds ranged from 29.30 – 35.10% with the highest of 35.10 at 15% and the lowest of 29.31% at 10% levels of spent yeast replacement. The red blood cells (RBC) ranged from 2.40 – 2.83 x 10^6/µl. The highest and lowest being 2.83 x 10^6/µl and 2.40 x 10^6/µl at 20 and 0% levels of spent yeast replacement respectively. The white blood cells (WBC) ranged from 2235.63 – 262.70 x 10^6/µl with the highest of 262.70 x 10^6 /µl being at 20% level of spent yeast replacement. The lowest (235.63 x 10^6 /µl) was at 5% level of replacement. The haemoglobin (Hb) ranged from 9.63 – 11.40 g/dl. The highest and lowest were 11.40 and 9.63 g/dl at 20% and 10% levels of spent yeast replacements respectively. The serum biochemical indices showed that total protein ranged from 4.65 – 5.90 g/dl. The highest (5.90 g/dl) was at 5% level of spent yeast replacement. The lowest (4.65 g/dl) was at 0% yeast level. Serum albumin which ranged from 1.45 – 2.08 g/dl showed highest value of 2.08 g/dl at 5% spent yeast replacement level while the lowest of 1.45 g/dl was at 15 and 20% spent yeast replacement levels. Serum globulin ranged from 3.20 – 4.43 g/dl, the highest of 4.43 g/dl being at 20% spent yeast replacement. The lowest (3.20 g/dl) was at 0% spent yeast level. Urea ranged from 4.00 – 8.67 mml/l, the highest being 8.67 mm/l at 10% and the lowest (4.00mm/l) at 20% spent yeast replacement levels respectively. Creatinine ranged from 105.00 – 164.00 mml/l. The highest (164.00 mmol/l) was at 10% and the lowest (105.00 mmol/l) were at 5% and 20% spent yeast replacement levels respectively. These results showed that all blood parameters were not significantly ($p > 0.05$) affected by the dietary treatments. **Conclusion:** Therefore replacement of soya bean by spent yeast up to 20% level has no adverse effect on blood parameters of broiler chickens and can be used.

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Introduction

It has been reported that feed accounts for not less than 70% of the total cost of production in livestock ventures. Therefore, there is the need to focus on cheaper sources of feed ingredients in order to maximize profits and avoid losses.

Spent brewers' yeast is a by-product of the brewery and distillery industries. It contains protein, amino acids and vitamins therefore can replace soya beans as protein source in the production of good quality poultry feeds. It is cheap and available and humans do not consume it. It is usually dumped in the environment as a waste product. This practice increases biological oxygen demand (BOD). Its use in poultry feed formulation will therefore also serve as a way of its disposal.

Yeasts have been used in feeds (Paryad and Mahmaudi, 2008; Huthail, 2014) as single cell proteins for their good protein, energy, amino acids, vitamins and crude fibre as well as metabolisable energy (ME) (Charlie, 2006).

The objective of this study is to assess the effect of graded levels of spent brewers' yeast on blood parameters of broiler chickens.

Materials and Methods

Experimental Diets

Five (5) isonootrognous starter and finisher diets were formulated for the study. Diet 1 was the formulated feed control containing 0% spent brewers' yeast. Diets 2, 3, 4 and 5 contained 5, 10, 15 and 20% spent brewers' yeast respectively, replacing soya bean cakes for starter and finisher feeds. The composition of the feeds are given in Table 1 based on calculated values.

Table 1: Composition of broiler starter and finisher feeds containing graded levels of spent brewers' yeast

Ingredients	**Starter**						**Finisher**				
	0 (%)	**5 (%)**	**10 (%)**	**15 (%)**	**20 (%)**		**0 (%)**	**5 (%)**	**10 (%)**	**15 (%)**	**20 (%)**
Maize	42.00	42.00	42.00	42.00	42.00		46.00	46.00	46.00	46.00	46.00
Wheat offal	10.87	10.87	10.87	10.87	10.87		12.82	12.82	12.82	12.82	12.82
Soya bean cake	40.33	35.33	30.33	25.33	20.33		33.88	29.05	24.00	19.00	14.59
Yeast	**0.00**	**5.00**	**10.00**	**15.00**	**20.00**		**0.00**	**5.00**	**10.00**	**15.00**	**20.00**
Fish meal	3.50	3.50	3.50	3.50	3.50		3.50	3.50	3.50	3.50	3.50
Bone meal	1.50	1.50	1.50	1.50	1.50		2.00	2.00	2.00	2.00	2.00
Lime stone	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25	0.25		0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25		0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10		0.10	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20		0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100		100	100	100	100	100
Calculated analysis											
CP (%)	23.00	23.00	23.00	23.00	23.00		21	21	21	21	21
ME (kcal/kg)	2990.54	2989.02	2962.12	2947.86	2972.91		2982.00	2985.00	2958.00	2950.00	2900.00
Ca (%)	1.18	1.18	1.18	1.18	1.18		1.20	1.20	1.20	1.20	1.20
P (%)	1.12	1.12	1.12	1.12	1.12		0.89	0.89	0.89	0.89	0.89

Key: CP = Crude protein; ME = Metabolizabale energy; Ca = Calcium; P = Phosphorus

Experimental Birds and Management

Two hundred 2 weeks old "sayed" birds were purchased at Bukuru market in Jos, Plateau State, Nigeria. The birds had been raised together for the first 13 days (adoption period) with a commercial feed (Vital Feed®) starter diet. The first dose of vaccine against Gumboro (infectious bursal disease) was administered on the 12th day at the hatchery. On day 14 of the age of birds, there were transported to the experimental site (Abubakar Tafawa Balewa University, Bauchi, Nigeria).

The birds were randomly picked and distributed into already labelled 20 partitions (pens) each measuring 1.4 x 2.2 m which consisted of 5 treatments with each treatment having 40 birds and these were further divided into 4 replicates of 10 birds each in a completely randomized design. Birds were individually weighed and recorded as initial weight in gram.

Isonitrogenous starter and finisher diets were supplied in each pen in an aluminium tube-type feeder. Water was supplied in a plastic 4-litre drinker. On this day the birds were fed with a mixture of 50% normal broiler starter diet (Vital Feed) and 50% experimental starter diet of each graded level as a means of gradually introducing the birds to the experimental diets. The birds were given anti-stress and glucose in their drinking water to tide them from the stress of transportation. An antibiotic (adamacine) was also given to prevent them from being infected by microorganisms which could come from the hatchery or during transportation. The birds were vaccinated against Newcastle disease on the 21st day of age and against the 2nd dose of Gumboro on the 28th day. The feeds and clean drinking water were provided *ad libitum*. All the birds were subjected to the same experimental and management conditions.

Feeds and water were checked in the mornings, afternoons and nights to ensure continuous supply. Starter diets were fed for the first 25 days after which finisher diets were fed for another 25 days. The experiment lasted for 50 days, 25 days each for starter and finisher phases.

Data Collection and Statistical Analysis

At the end of the experiment, five (5) birds per treatment (that is, 1 bird per replicate) having their weights closest to the average of their group were selected. Blood was collected from 4 randomly selected birds per treatment for the determination of haematological and serum biochemical parameters. According to Bush (1975), samples were collected from the bronchial vein using 5ml disposable syringes and needles (21 gauge). Blood samples were analysed for haematological parameters according to routinely available clinical methods described by Baker *et al.* (1998).

Determination of Packed Cell Volume

The packed cell volume was determined using Wintrobes micro-haematocrit method. Fifty (50) microliter capillary tubes and microcentrifuge were

used. An uncalibrated capillary tube, 75 cm long and 1 mm in diameter was used. The blood samples were gently mixed in the collection tubes and poured into the capillary tubes. The blood entered the tubes by capillary attraction and was removed when two-thirds of it was filled with blood.

One end of the tube was sealed by a hot flame and then placed with the sealed end outwards in the centrifuge. This was spun at 2000 revolutions per minute for 5 minutes to allow the centrifugal force to separate the blood into cells and plasma. The PCV for each sample was read using Hawsley microhaematocrit reader (Gelman-Hawksley, Limited, UK) graded in percentages (Aluwong *et al.* 2012).

Determination of White Blood Cells (WBC) or Leucocytes

The improved Neubauer Haemocytometer method was used for the determination of leucocyte counts. Blood was drawn to the 0.5 unit mark on the stem of the white cell pipette and diluting fluid to the 1 unit mark. This produced a dilution of 1:2. The WBC diluting fluid (Turk's Solution) contained 1% glacial acetic acid, which destroyed the erythrocytes, tinged with gentian violet (0.01%) which stained the leucocytes. Up to half the pipette content was blown out and discarded to expel the cell-free fluid from the pipette.

The cells in the 4 corner primary squares were counted using the Neubauer Chamber. The X10 objective and X10 eyepiece of the microscope were used in the counting. The white cell count was obtained by calculation according to Baker *et al.*, (1998).

Let N = Number of cells counted in $4mm^2$

Depth of the chamber = 1/10 mm

N cells are counted in 4/10 mm^3 (i.e. 4 × 1/10 mm^3) of diluted blood

$1mm^3$ of diluted blood contains N × 10/4 cells

Blood dilution in the ratio of 1:20 ($^{v}/_{v}$)

$1mm^3$ of blood contains $N \times {}^{10}/_{4} \times {}^{20}/_{1} = 50 \times N$ cells

Determination of Haemoglobin (Hb) Concentration

The Hb concentration of the samples was estimated using the acid haematin and Sahli method. The blood was diluted in a solution of HCL, which converted haemoglobin to haematin. The graduated tube was filled to the 20-unit mark with 0.1N HCL. About 0.02 cm^3 of blood was added, mixed well and allowed to stand for 10 minutes. The acid (0.1 NHCL) was added drop-by-drop mixing between each addition until the colour matched the standard.

The amount of solution in the graduated tube was read on the calibration, which gave the haemoglobin concentration as a percentage (%). The percentage was converted to g/l by multiplying the values by 1.46 (WHO, 1999).

Determination of Serum Biochemical Indices

Serum total protein, albumin, globulin, creatinine and urea were analyzed using sigma assay kits (Sigma Chemical Co., St. Louis, Missouri, USA). The total protein and serum albumin were determined by Biuret reactions (Bush, 1975). The total protein was first estimated and then performing fraction on further volume of the sample to precipitate and remove globulins leaving albumin in solution.

Statistical Package for Social Sciences (SPSS) Software, version 23 for Windows (IBM, USA) was used to analyze and the data obtained were expressed as mean. Data collected were subjected to analysis of variance (ANOVA) and the difference among means were compared using Duncan's Multiple Range Test. Values of $p<0.05$ were considered significant. (Steel and Torrie, 1980).

Results and Discussion

The haematological parameters are shown in Table 2. The packed cell volume of the broiler chickens in this study ranged from 29.33 – 35.10%. This was in agreement with published values of 28 – 40% by Swenson (2004). Yisa (2011), which was 35.10% was at 15% spent yeast level while the lowest (29.33%) was at 10% level. There was however, no significant difference ($p > 0.05$) between the treatment levels. This result also compares with what was reported by Ameen *et al.* (2007) and Iyali *et al.* (2008). The red blood cells (RBC) ranged from 2.40 – 2.80 x 10^6 /µl. Yisa (2011) reported a range of 3.88 – 5.05 x 10^6 /µl. However, the results contained in this study compared with the standard which is 2.5 – 3.2 x 10^6 /µl. There was no significant difference ($p > 0.05$) between the treatment levels.

The white blood cells ranged from 235.63 – 262.70 x 10^6 /µl with the highest of 262.70 x 10^6 /µl being at 20% level of spent yeast replacement. The lowest (235.63 x 10^6 /µl) was at 5% level of replacement. Yisa (2011) published a range of 3.23 – 3.40 x 10^9 /µl and this agrees with the range obtained in this work. There was however no significant difference ($p > 0.05$) between the treatments.

The haemoglobin ranged from 9.63 – 11.40 g/dl with the highest of 11.40 g/dl being at 20% level of spent yeast replacement. The lowest which was 9.63 g/dl was at 10% level. This result agrees with the findings of Swenson (2004) and Akinmutimi (2004) who got 9.0 g/dl and 9.75 – 12.27 g/dl respectively. Yisa (2011) got a range of 10.40 – 11.53 g/dl for broiler chickens. The results in this work showed that all the haematological parameters of broiler chickens fed the trial diets were highest at 20% spent yeast level except for PCV which was highest at 15% level of spent yeast replacement.

Table 2: Haematological parameters of broiler chickens fed on feeds containing graded levels of spent brewers' yeast (*Saccharomyces cerevisiae*) at 50 days

Parameter	Level of spent yeast replacement (%)					
	0	5	10	15	20	P value
PCV (%)	31.40	30.33	29.33	35.10	34.73	0.695
RBC ($x10^6/\mu l$)	2.40	2.44	2.44	2.79	2.83	0.738
WBC ($x10^6/\mu l$)	255.90	235.63	251.60	259.63	262.70	0.746
Hb (g/dl)	10.17	9.67	9.63	11.37	11.40	0.659

Key: PVC = Packed Cell Volume; RBC = Red Blood Cell; WBC = White Blood Cells
HB = Haemoglobin

The serum biochemical indices of broiler chicken showed in Table 19 that serum total protein ranged from 4.65 – 5.90 g/dl. The highest which was 5.90 g/dl at 5% level of spent yeast replacement. The lowest which was 4.65 was at 0% level of spent yeast inclusion. There is no clear explanation for this. However, the values were higher than those obtained by Paryad and Mahmoudi (2008) who obtained a range of 3.19 – 4.00g/dl (Table 3). The result obtained in this work was not significantly different (P>0.05) between the treatments and compared with the standard which is 4.0 – 8.4 g/dl.

Serum albumin which ranged from 1.45 – 2.08 g/dl showed highest value of 2.08 g/dl at 5% spent yeast level while the lowest of 1.45 g/dl was at 15 and 20% spent yeast levels. These results also compared favourably with those obtained by Paryad and Mahmoudi (2008) but slightly higher. There was however no significant difference (P>0.05) between the treatments and compared with the standard of 2.1 – 3.4 g/dl.

Table 3: Serum biochemical indices of broiler chickens fed on feeds containing graded levels of spent brewers' yeast (*Saccharomyces cerevisiae*) at 50 days

Parameter	Level of spent yeast replacement (%)					
	0	5	10	15	20	P value
Serum total protein (g/dl)	4.65	5.90	5.00	5.48	5.88	0.205
Serum Albumin (g/dl)	1.48	2.03	1.70	1.45	1.45	0.095
Serum Globulin (g/dl)	3.20	3.88	3.30	4.03	4.43	0.205
Urea (mmol/l)	5.80	6.25	8.67	7.85	4.00	0.024
Creatinine (mmol/l)	120.00	105.00	164.00	141.00	105.00	0.424

Serum globulin ranged from 3.20 – 4.43 g/dl. The highest which was 4.43 g/dl was at 20% spent yeast level while the lowest of 3.20 g/dl was at 0% spent yeast level. These results were much higher than those obtained by Paryad and Mahmoudi (2008) which ranged from 1.63 – 1.89 g/dl. Urea ranged from 4.00 – 8.67 mmol/l with the highest of 8.67 mmol/l being at 10% spent yeast level while the lowest (4.00 mmol/l) was at 20% level. Urea was significantly affected (P < 0.05) by the dietary treatments.

Creatinine ranged from 105.00 – 164.00 mmol/l. The highest value was at 10% spent yeast replacement while the lowest of 105.00 mmol/l was at 5 and 20% spent yeast levels. The results do not show a consistent pattern of variation and the reasons are not clear. However, results of the serum biochemical indices compared favourably well with the standards and in some cases even better. All except urea were not significantly different (P > 0.05) between the treatments. Urea was however, significantly different (P<0.05).

Conclusion

Therefore replacement of soya bean with spent brewers' yeast at the levels tested do not affect the blood of the birds adversely. Up to 20% replacement was used in this study without any adverse effects on the blood parameters of broiler chickens.

Corresponding Author:
Dr. Patricia Fremu Chollom,
Department of Microbiology,
Faculty of Natural Sciences,
University of Jos, Jos, Nigeria.
Telephone: +2348034917313
E-mail: ciachollom@yahoo.com

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