Haematological and serum biochemical indices of broiler chickens fed roselle seed (*Hibiscus sabdariffa* L.) cake based diet as replacement for soyabean meal

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Abstract

Feeding trial experiment was carried out to evaluate the haematological and serum biochemical parameters of broiler chickens fed graded levels of Roselle Seed Cake (RSC) as replacement of a conventional protein source. Proximate composition was done to determine the nutrient content of RSC and experimental diets. The experiment contained graded levels of RSC replacing soya bean cake (SBC). One hundred and twenty (120) day old Ross broiler chickens were used in a Completely Randomized Design (CRD). The broiler chicks were assigned to five dietary treatments with three replicates each with 8 birds per replicate and 24 birds per treatment. Five isonitrogenous and isocaloric diets were formulated for the broiler straight diet. Diet 1 served as the control diet while diets 2, 3, 4 and 5 contained 8%, 16%, 24% and 32% RSC respectively. Data were collected and analyzed using analysis of variance and the mean was separated using Duncan multiple range test. The findings from the study showed that there were no significant ($P=0.05$) differences in all the parameters measured for haematology across dietary treatment means except for the white blood cell. The blood biochemical composition showed no significant ($P=0.05$) differences in total protein, high density lipoprotein, low density lipoprotein and creatinine across dietary treatments. RSC as an alternative feedstuff can replace soya bean cake at 24% in the diet of broiler chickens for improved haematological and blood biochemical of broilers. Roselle seed cake can replace SBC at the broiler chicken straight ration at 75% (24% dietary RSC) inclusion for better performance.

Keywords: Rosselle seed cake, Soyabean meal, haematological, serum biochemical

Introduction

Feed cost and continuous effort to get better understanding of the feeding value of available alternative feed ingredients has been a major challenge in the poultry industry in Nigeria. Inadequate knowledge and non-availability of alternative and cheaper feed resources and competition between man and animals for most of the conventional feedstuffs have been identified as the root cause (Afolayan *et al.*, 2012). Protein is a very important nutrient that is responsible for growth and its deficiency leads to growth depression. Proteins are used in the construction of body tissues such as muscles, nerves, cartilage, skin, feathers, beak, and so on. All animals require protein for growth, reproduction and production depending on the age, productivity of the animal and their environment (Olomu, 1995).

The livestock industry is having difficulties in meeting the demand for animal feeds which accounts for about 70% of the cost of

production in animal production enterprise. The Nigerian livestock industry is operating below its capacity because of the high cost and scarcity of feed resources (Aduku and Olukosi, 1990). Affordability and availability of conventional feed sources have been a challenge in the poultry industry and the ever rising pressure on legumes such as soya beans particularly as the major sources of protein in poultry feeds has been more challenging. Most of the feed sources used in poultry industry such as maize, soya bean, and groundnut are also being consumed by humans. Therefore, it is necessary to look for alternative feed sources which could be incorporated into poultry diet. Several legumes have been investigated for their potentials as alternative protein feedstuffs in poultry nutrition. Roselle seeds for instance have been investigated for their nutritional value in poultry while the cake have been studied for feeding value with and without amino acid supplementation in poultry

nutrition (Igoche, 2015). However, the effect of the cake as replacement for conventional protein ingredients like soya bean cake (SBC) has received little attention. The cake can be a potential alternative protein ingredient in poultry diets. Roselle seedcake (RSC) is not consumed by humans, it is cheap and readily available therefore pose no threat of scarcity. This study was designed to evaluate the effect of graded levels of Roselle seed cake as an alternative protein source as replacement of conventional protein source in broiler diets.

Materials and methods

The experiment was carried out at the Poultry Unit of the Teaching and Research Farms of the Department of Animal Science, Nnamdi Azikiwe University, Awka, Nigeria which is within the forest vegetation zone of southeastern part of Nigeria in Anambra state's capital Awka of latitude 6.2437°N and longitude of 7.1219°E with elevation of 415 feet and eye altitude of 75m-77m above sea level. This area lies in the tropical climate with mean average rainfall of 1.037mm and a mean ambient temperature of about 34.7°C and yearly average humidity of 83% (Ezenwaji *et al.*, 2013).

Roselle seedcake, soya bean cake and all other ingredients used for the experiment were purchased from Eke Awka in Anambra State. Samples of the Roselle seedcake and experimental diets were taken to the Animal Science Biochemical Laboratory for proximate

analysis using the methods described by A.O.A.C (2005). The analysis was done to obtain the nutrient composition (Dry matter, Ash, ether extract, crude protein, crude fibre and nitrogen free extract) of the experimental diet. The metabolizable energy was determined using the Janssen formula (Janssen, 1989). $ME_n = 29.68 \times DM + 60.95 \times EE - 60.87 \times CF$. Five isonitrogenous and isocaloric diet (23.33 %CP; 3001kcal/kg ME) was formulated for the broiler chickens as shown in Table 1

One hundred and twenty (120) day old Ross broiler chicks purchased from a reputable farm were used for the study. The broiler chicks on arrival were counted and were given a common ration for one day and allowed to adapt to the environment. Their initial average weight was recorded and the chicks were randomly distributed into five treatment groups of 24 chicks each with three replicates of 8 chicks per pen in a Completely Randomized Design. (CRD). Each group was assigned to the five experimental diets and they were all subjected to the same management practices all through the 56 days period of the experiment. The birds were given lasota Intra ocular (I.O) vaccine on arrival and vitalityte was added to their water to reduce stress. They were given their first dose of gumboro at 7 days old, first dose of lasota at 14. days old while second dose of gumboro and second dose of lasota were administered at 21 days and 28 days old respectively. The birds were also treated against coccidiosis at 3weeks of age and the mode of administration was oral.

Table 1: Gross composition of experimental straight diets for broiler chickens with Roselle seed cake as replacement for soya bean cake

Ingredients (%)	Levels of Roselle Seed Cake (RSC) Inclusion (%)				
	0	25	50	75	100
Maize	61.36	57.05	54.30	51.94	48.85
Roselle seed cake	0.00	8.00	16.00	24.00	32.00
Soya bean cake	19.70	16.00	10.70	5.00	0.00
Groundnut cake	11.00	11.00	11.00	11.00	11.00
Blood meal	4.00	4.00	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.20	0.20	0.20	0.20	0.20
Common salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.04	0.05	0.10	0.16	0.20
*Vitamin premix	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated Analysis					
ME (Kcal/kg)	3001.00	3002.00	3019.00	3037.00	3050.00
Crude protein (%)	23.33	23.30	23.32	23.34	23.36

Vit A, 10,000IU; Vit D3, 2,000 IU; Vit E, 23,000mg; Vit K3, 2,000mg; Vit B1, 1,800; VitB2, 5,500mg; Niacin, 27,500; Panthoenic acid, 7,500; Vit B6(pyridoxine) 3,000mg; Vit B12, 15mg; Folic acid, 750mg; Biotin H2, 60mg; Cholin Chloride, 300,000mg; Cobalt, 20 0mg; Copper,3.0mg; Iodine,3,000mg;Iron,1000.00mg;Manganese,20,000mg; Selenium, 40,000mg; Zinc, 200mg; Antioxidant ,1,250mg , ME= Metabolizable Energy

Two millilitres (2.0mls) of blood were collected from the wing vein of each bird at the end of the growth phase. One bird per replicate making a total of three birds per treatment were randomly selected. Blood samples were collected into labelled bottles containing Ethylene Diamine Tetra acetic Acid (EDTA) and taken to the Nutrition and Biochemistry Laboratory for haematological analysis. The samples were assayed for: Packed cell volume (PCV), haemoglobin content (hb), white blood cell (WBC), red blood cell (RBC) and differential blood count (heterophils, lymphocytes, monocytes, eosinophils, basophils and band cell). The haematological analysis was performed using a semi-automatic cell counter (Sysmex-F820). Blood smears was ethanol-fixed and stained according to the May-Grunwald-Giemsa technique. The blood smears was used to determine the differential blood count by immersion microscopy and quantified by immersion refractometry. Blood samples were analyzed for total protein, total cholesterol, tryglycerides, high density lipo protein (HDL), low density lipoprotein (LDL), albumin and creatinine. Analysis was done at the Nutrition and Biochemistry Laboratory. The total blood protein concentration was determined based on the biuret reaction (Dioumas *et al.*, 1981) and albumin determination as described by Doumas and Biggs (1972). Creatinine determination was done according to the method by Ambrose *et al.*, (1983). Total cholesterol levels were estimated using the Liebermann Buchard reaction according to the principles of Huang *et al.* (1961) and modified by Ness *et al.* (1964). Tryglycerides,

HDL and LDL were determined using spectrophotometer.

The data obtained from the study were analyzed using One-way ANOVA. The General Linear Model procedure of Statistical Analysis System (SAS, 2002) was used and the mean was separated using Dunnet because the control diet was used to compare with other treatments (Dunnet, 1964).

Results and discussion

The proximate composition of Roselle Seed Cake is shown in Table 2. The crude protein value of 30.45% observed for Roselle seed cake in this study is similar with the findings of Igoche (2015) who reported that the crude protein of RSC is 30.50 and it agrees with the findings of Isidahomen *et al.* (2006) and Kwari *et al.* (2011) that Roselle seed cake contains between 21.40-38.75% crude protein. Crude fibre value of 4.20% obtained was lower than the value 11.98% reported by Mukhtar (2007) and also 10.20% reported by Sanni, (2015) but similar to the value 4.18% reported by Igoche (2015). Ether extract value of 8.05% was quite lower than the value of 17.43% reported by Mukhtar (2007) and higher than 5.78% reported by Sanni, (2015). However, it was closer to the value of 10.28% reported by Igoche (2015). The metabolizable energy value of 3561.92kcal/kg agreed with the range of 2880-3500kcal/kg reported by various authors (Isidahomen *et al.*, 2006; Mukhtar, 2007; Diarra *et al.* (2011) and Kwari *et al.* (2011). These variations in values obtained could be attributed to different sources of the cake and differences in processing methods employed and storage conditions.

Table 2: Proximate composition and energy value of Roselle seed cake

Parameters	(%)
Dry matter	95.17
Crude protein	30.45
Crude fibre	4.20
Ash	7.25
Ether extract	8.05
Nitrogen Free Extract	50.05
ME(Kcal/kg)	3059.64

The result of the haematological indices of broiler chickens fed graded levels of Roselle seed cake as replacement for soya bean cake is presented in Table 3. The findings from this study showed no significant ($P=0.05$) differences in the packed cell volume, haemoglobin (Hb) and red blood cell across dietary treatments except for the white blood cell which was observed to show some significant ($P=0.05$) differences. This result is similar to the findings of Igoche, (2015) who reported no significant differences in packed cell volume, haemoglobin and total protein in broilers fed RSC with or without amino acid supplementation. The values for WBC was quite higher than the normal range in all the treatments. The white blood cell provides the body's defense as part of the immune system and high WBC count indicates diseases or stress in the birds (Peter, 2002). High mortality results to high WBC and lymphocytes. It was observed in this study that the high values of WBC had the same trend with the mortality rate of birds in the different groups. The differential blood counts which are the heterophils, lymphocytes, monocytes, eosinophils and basophils were not significantly ($P=0.05$) different from each other across dietary treatments. These differential counts are the different types of white blood cells found in the birds and the values obtained is an indication that the health challenge of the birds was not as a result of the test diet.

The result of blood biochemical parameters of birds fed graded levels of Roselle seed cake as replacement from soya bean cake is shown in Table 4. There were no significant ($P=0.05$) differences in total protein, high density lipoprotein and creatinine across dietary treatment means. Total cholesterol level and low density lipoprotein were observed to have a non-significant ($P=0.05$) values with birds on the 16%, 24% and 32% RSC diets. Tryglycerides level at 8% and 16% RSC inclusion were not significantly ($P=0.05$) with birds on the control diet and lower with birds on 24% and 32% RSC inclusion. The albumin concentration was significantly ($P=0.05$) higher with birds on the 8% and 24% inclusion compared to the rest dietary treatments. From the results obtained, it was observed that total cholesterol level and tryglycerides increased linearly as the inclusion of RSC increased but was still within the normal range. This increase might be that higher inclusion level of RSC can increase the fat content in the birds. Creatinine is a nitrogenous waste product formed from the metabolism of creatinine in skeletal muscle. Measurement of creatinine is used as a test of kidney function and high level signifies impaired kidney function. The normal range is 0.2-0.5mg/dl (Peter, 2002). The values obtained in this study (0.47-0.50mg/dl) is an indication that RSC had no detrimental effect on the birds kidney function and the RSC did not result in muscle wasting in the birds (Cheesbrough, 1987).

Table 3: Haematological parameters on broiler chickens fed graded levels of Roselle seed cake as replacement for Soya bean cake

Parameters	Levels of Roselle Seed Cake (RSC) Inclusion (%)					SEM
	0	8	16	24	32	
PCV (%)	23.33	26.00	28.67	28.33	29.00	3.92
Hb (g/dl)	7.77	8.63	9.53	9.43	9.63	1.31
RBC (×106/dl)	3.90	4.10	4.63	4.00	4.07	0.68
WBC (×103/ml)	10.40 ^{ab}	12.27 ^{ab}	9.27 ^{ab}	7.73 ^b	15.47 ^a	2.01
Heterophils (%)	8.67	10.67	16.33	13.67	9.67	3.52
Lymphocytes (%)	90.33	85.33	82.00	86.33	90.33	2.99
Monocytes (%)	1.00	1.33	0.67	0.00	0.00	0.80
Eosinophils (%)	0.00	1.00	0.00	0.00	0.00	0.45
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00
Band cell (%)	0.00	1.67	1.00	0.00	0.00	0.47

ab= Means with different superscripts on the same row are significantly different $P \leq 0.05$

PCV: Packed cell volume, Hb: Haemoglobin, WBC: White blood cell, RBC: Red blood cell
SEM= Standard Error of Mean

Table 4: Blood biochemical parameters of broiler chickens fed graded levels of Roselle seed cake as replacement for soya bean cake

Parameters	Levels of Roselle Seed Cake (RSC) Inclusion (%)					SEM
	0	8	16	24	32	
Tryglycerides (g/dl)	63.67 ^b	67.00 ^b	69.33 ^b	75.67 ^a	74.00 ^a	2.97
Total cholesterol(mg/dl)	114.67 ^c	120.00 ^b	124.67 ^a	125.67 ^a	128.33 ^a	2.32
Total protein(g/dl)	3.70	3.77	4.07	3.47	3.57	0.40
HDL (mg/dl)	86.33	66.67	74.67	79.00	86.33	14.08
LDL (mg/dl)	44.30	44.20	44.35	44.33	44.42	0.35
Albumin (mg/dl)	28.00 ^b	34.00 ^a	24.67 ^b	35.67 ^a	28.33 ^b	2.32
Creatinine (mg/dl)	0.48	0.47	0.50	0.47	0.49	0.23

abc= Means with different superscripts on the same row are significantly differentP= 0.05

HDL: High density lipoprotein, LDL: Low density lipoprotein

SEM= Standard error of mean

Conclusion

Based on the findings of this study, it was concluded that Roselle seed cake (RSC) as an alternative feedstuff did not have adverse effect on the haematology and blood biochemical of broilers birds, therefore can replace soya bean cake (SBC) at 75% (i.e 24% dietary roselle seed cake) inclusion levels and incorporated in the diet of broiler chickens.

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