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Heamatological and biochemical indices of broiler chickens fed graded levels of boiled sorrel (*Hibiscus sabdariffa*) seed meal as a replacement for soybean

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ABSTRACT: Escalating feed costs have significantly impacted the poultry industry in developing countries like Nigeria, constituting a substantial portion of total expenses. This has motivated the exploration of unconventional and cost-effective feed ingredients like sorrel (*Hibiscus sabdariffa*) seed meal. This study was conducted to assess the heamatological and biochemical indices of broiler chickens when fed varying levels of boiled sorrel Seed Meal (BSSM). One hundred and thirty-five-day old Anak broiler chicks were utilized in a completely randomized design (CRD). The chicks were divided into three dietary groups, each with three replicates containing 15 birds per replicate, totaling 45 birds per treatment. During the starter and finisher phases, three diets were formulated, incorporating boiled sorrel seed meal to replace soya bean meal at levels of 10% and, 20% respectively. At six weeks of age, blood samples were collected via venipuncture of the wing vein using a 3 ml syringe and 23-gauge needle. The samples were immediately separated into two tubes: one containing ethylene diamine tetra-acetic acid (EDTA) for heamatological analysis and the other without anticoagulant for serum biochemical analysis. The heamatological parameters were determined using an automatic fully digital heamatology analyzer (Boevopeak Ltd, China), while biochemical parameters were determined by universal clinical auto analyzer (Boevopeak Ltd, China). Results of heamatological indices revealed no significant differences in packed cell volume (PCV), heamoglobin concentration (Hb), and red blood cells (RBC). Differential blood counts did not show significant differences except for lymphocyte levels which displayed some significance. Serum biochemical parameters including total protein (albumin and globulin), triglycerides, and glucose did not exhibit significance. However, alanine transaminase levels showed significance with the inclusion of sorrel seed meal.

Keywords: Biochemical indices, broiler chicken, heamatological indices, sorrel seed.

INTRODUCTION

Over the past decade, the poultry industry in Nigeria has faced with the significant impact of soaring feed costs, constituting a substantial portion of total expenses (Uchegbu *et al*., 2010). Livestock production in developing countries, like Nigeria, witnesses feed expenses accounting for 60-80% of overall costs (Igboefi and Esonu, 2000). To address this challenge, Nigerian livestock farmers are increasingly exploring unconventional feed ingredients, such as sorrel seed meal, derived from *Hibiscus sabdariffa I*, locally known as "*Yakuwa*" in Nigeria. *Hibiscus sabdariffa* is a well-adapted crop in the semi-arid zone of West Africa, including Nigeria, commonly cultivated as a border crop. Its leaves are utilized as a vegetable, while the floral parts are employed

in preparing the local drink known as "*Zobo*." The stem provides fiber, and the seeds serve as a source of nutrition for scavenging poultry (Philips, 1977). Unfortunately, large quantities of these seeds go to waste annually, with minimal collection and storage for planting. To address the challenges posed by the high costs of conventional feed ingredients like cotton seed cake (CSC), soybean meal (SBM), and groundnut cake (GNC), there is a growing interest in exploring alternative protein sources. This shift could not only maximize returns for poultry enterprises but also reduce overall feed costs. The overreliance on soybean meal and groundnut cake as primary protein concentrates in Nigeria and other developing countries poses a considerable hindrance to the progressive growth of the livestock industry. Concerns about compromised standards among commercial feed operators, as evidenced by discrepancies between composition labels and actual nutrient content on branded feed bags, further underscore the need for change (Kudu *et al*., 2008). In this context, nutritionists are urged to investigate the potential of incorporating cost-effective protein sources, such as sorrel seed meal into poultry diets. *Hibiscus sabdariffa* is recognized for its rich nutritional profile, including proteins, dietary fiber, carbohydrates, and fats (El-Adawy and Khalil, 1994; Rao, 1996; Abu Tarboush *et al*.,1997). Additionally, these seeds boast high mineral content, comprising calcium, magnesium, and phosphorus. Reports from Nigeria indicate that the seeds contain approximately 35-90% crude protein, 10.14% ether extract, 10.09% ash, and 15-17% crude fiber (Dashak and Nwanegbo, 2002; Kwari *et al*., 2011). Despite their nutritional benefits, unprocessed sorrel seeds contain anti-nutritional factors like total phenols, tannins, and phytic acid, which can harm animal health and performance, but various processing methods, such as boiling water treatment, fermentation, and ice water treatment, have been explored to mitigate these anti-nutritional factors (Obadire *et al.,* 2022). Blood constituents play a crucial role in understanding the physiological and pathological conditions of animals (Etim *et al*., 2014). Evaluating the blood profile of poultry provides insights into how dietary treatments meet their metabolic and health requirements (Olafadehan *et al*., 2014). Deviations from normal blood values can indicate the animal's metabolic state and the quality of feed, reflecting its systemic relationship and physiological adaptation to environmental, nutritional, or pathological stressors (Wheater *et al*., 2018). The use of biochemical indices as a pointer to conditions that may not be readily noticed by performance indices cannot be overemphasized; plane of nutrition is known to affect these values (Agunbiade *et al*., 2007). This assessment is particularly important for non-conventional feed (NCFs) ingredients, as their value depends on their nutritive content, availability, and safety for animal health (Igoche, 2015). In this context, this research was aimed at evaluating the effect of boiled sorrel seed meal (BSSM) as replacement for conventional feed ingredients like soybean meal (SBM) on heamatological and serum biochemical indices of broilers chicken.

MATERIALS AND METHODS

Experimental site

The research was conducted at the Federal University Wukari Teaching and Research Farm in Wukari Local Government Area, Taraba State. The study area, situated between latitude 9.45° and 7.87° N and longitude 9.7° and 9.79° E, falls within the northern guinea savanna zone with a tropical wet and dry climate. The dry season spans a minimum of five months (November to March), followed by the wet season from April to October. The region experiences an annual rainfall ranging from approximately 223 to 1205 mm. Relative humidity varies from 5-39%, and the average temperature is around 39°C (Taraba state Government Diary, 2018).

Hibiscus sabdariffa source and processing

Sorrel seeds, alongside other feed ingredients like maize, groundnut cake, and soya bean, were procured from the Wukari market, Nigeria. Lysine and methionine were obtained from Alheri Agro-Vet store in Jalingo, Taraba State Nigeria. Water was boiled until it reached its boiling point, after which sorrel seeds were immersed in the hot water for approximately 30 minutes. The seeds were then extracted, dried for three days, ground, and subsequently incorporated into the diets.

Experimental diets, design, and animal management

Diets were formulated with *Hibiscus sabdariffa* seeds at 0, 10, and 20% for starter and finisher diets (T_1, T_2, and T_3) as outlined in Tables 1 and 2. Treatment one (T_1) served as the control diet. The experiment utilized a completely randomized design, involving a total of one hundred and thirty-five (135) day-old broiler chicks. After one week of brooding, the chicks were weighed, randomly assigned to three dietary treatments (45 broiler chicks per treatment), and replicated three times, with fifteen birds per replicate. The feeding trial spanned six weeks, during which the birds were managed in a deep litter system. *Ad libitum* access to drinking water and feed was provided, and routine and occasional management practices were conducted following recommendations of Roberts (2000).

Collection and analysis of blood samples

At the end of the 6th week, birds were subjected to 12 hours fasting prior to slaughtering and blood collection.

Table 1. Ingredients composition of broiler starter diet (1-3weeks)

Ingredients	Replacement levels of boiled sorrel seed meal (BSSM)		
	T_1 (control, 0%)	T_2 (10%)	T_3 (20%)
Maize (white)	55.00	55.00	55.00
Soybean meal	30.00	27.00	24.00
Groundnut cake	10.00	10.00	10.00
Boiled sorrel seed meal	00.00	3.00	6.00
Limestone	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00
Premix	0.30	0.30	0.30
Lysine	0.20	0.20	0.20
Methionine	0.20	0.20	0.20
Salt	0.30	0.30	0.30
Total	100	100	100
Calculated analysis			
Crude protein	22.80	22.67	22.50
Crude fiber	4.30	4.40	4.50
Ether Extract	4.70	4.75	4.80
Calcium	1.23	1.22	1.22
Phosphorous	0.52	0.52	0.52
ME Kcal/kg	2856.00	2868.00	2879

ME= Metabolizable Energy (kcal/kg); BSSM= Boiled Sorrel Seed Meal; *Vitamin-Mineral premix provides per kg the following: Vit. A 1500 IU; Vit D3 3000 IU; Vit.E; 30 IU; Vit.K 2.5mg; Thiamine B_1 3mg; Riboflavin B_2 6mg; pyridoxine B_6 4mg; Niacin 40mg; Vit. B_{12} 0.02mg; pantothenic acid 10mg, Folic acid 1mg; Biotin 0.08g; chloride 0.125g; Mn 0.096g; Antioxidant 0.125g; Zn 0.06g; Fe 0.024g; Cu 0.006g; 10.0014g; Se 0.24g; Co 0.240g.

Table 2. Ingredients composition of broiler finisher diet (4-6weeks).

Ingredients	Replacement levels of boiled sorrel seed meal (BSSM)		
	T_1(control, 0%)	T_2 (10%)	T_3 (20%)
Maize (white)	60.00	60.00	60.00
Soybean meal	25.00	22.50	20.00
Groundnut cake	10.00	10.00	10.00
Boiled sorrel seed meal	00.00	2.50	5.00
Limestone	1.00	1.00	1.00
Bone meal	3.00	3.00	3.00
Premix	0.30	0.30	0.30
Lysine	0.20	0.20	0.20
Methionine	0.20	0.20	0.20
Salt	0.30	0.30	0.30
Total	100	100	100
Calculated analysis			
Crude protein	21.04	20.14	20.80
Crude fiber	4.00	4.08	4.16
Ether Extract	4.56	4.60	4.64
Calcium	1.22	1.21	1.21
Phosphorous	0.51	0.51	0.51
ME Kcal/kg	2908.00	2918.00	2928.00

ME= Metabolizable Energy (kcal/kg); BSSM= Boiled Sorrel Seed Meal; *Vitamin-Mineral premix provides per kg the following: Vit. A 1500 IU; Vit D3 3000 IU; Vit.E; 30 IU; Vit.K 2.5mg; Thiamine B_1 3mg; Riboflavin B_2 6mg; pyridoxine B_6 4mg; Niacin 40mg; Vit. B_{12} 0.02mg; pantothenic acid 10mg, Folic acid 1mg; Biotin 0.08g; chloride 0.125g; Mn 0.096g; Antioxidant 0.125g; Zn 0.06g; Fe 0.024g; Cu 0.006g; 10.0014g; Se 0.24g; Co 0.240g.

Three birds from each replicate (making a total of 54 birds) were randomly selected from the three treatments. Fifty-four blood samples (4 ml each) were collected from the birds by venipuncture of the wing vein using a sterile syringe and needle and put in ethylene diamine tetra-acetic acid (EDTA) treated bottles. The samples were quickly stored in an ice box, using icepacks and transferred to the laboratory for heamatological and serum biochemical analysis, within three hours' post sampling. An Automatic Fully Digital Heamatology Analyzer (HEMAD6031, Bioevopeak Ltd, China) was used for determination of Heamoglobin, Hb (g/dl), Packed Cell Volume, PCV (%), Red Blood Cell, RBC (X106 /µl) and White Blood Cell, WBC (X103 /µl). The Universal Clinical Auto Analyzer (Boevopeak Ltd, China) was used for serological determination.

Data analysis

Data generated were subjected to one-way analysis of variance (ANOVA) (SAS, 2009). Significant means at 5% level of probability was separated using Duncan's Multiple Range Test of the same statistical package.

RESULTS AND DISCUSSION

The ingredient and calculated nutrient compositions of the broiler starter and finisher diets containing 0, 10, and 20% boiled sorrel seed meal (BSSM) are presented in Tables 1 and 2, respectively. The calculated crude protein (CP) levels in the starter diets (Table 1) were 22.8, 22.67 and 22.5% for the control, 10% BSSM and 20% BSSM diets, respectively. These values are similar to the 22-23% CP recommended for broiler starters by Oluwafemi (2020). However, Angbulu *et al*. (2020) reported slightly higher CP levels of 23.25-24.11% when including up to 15% fermented roselle seed meal in broiler starter diet.

In the finisher diets (Table 2), the CP decreased from 21.04% in the control to 20.14% with 10% BSSM and 20.8% with 20% BSSM inclusion. These CP levels are within the 22.5% recommended for broiler chickens by Akinmutimi *et al*. (2018). Onunkwo *et al*. (2019) also reported a CP range of 19.76-20.95% when roselle seed cake replaced 0-20% of soybean meal in broiler finisher diets.

The metabolizable energy (ME) values increased with higher BSSM inclusion in both starter (2856-2879 kcal/kg) and finisher (2908-2928 kcal/kg) diets in this study. This agrees with Nyame *et al*. (2022) who found ME values of 2900.02-2941.49 kcal/kg roselle seed meal replaced up to 20% soybean meal in broiler finisher diets.

The crude fiber levels ranged from 4.3-4.5% in the starter diets and 4.0-4.16% in the finisher diets, which are below the 7-9% range recommended for poultry (Zhang *et al*., 2023). Wafar (2013) reported similar CF values of 3.96-4.63% when toasted sorrel seed meal replaced soybean meal in broiler finisher diets.

The results for the proximate composition of boiled sorrel seed meal (BSSM) and raw sorrel seed meal (RSSM) are presented in Table 3. BSSM exhibited a Dry Matter (DM) content of 92.7%, Crude Protein (CP) of 30.85%, Crude Fiber (CF) of 5.28%, Ether Extract (EE) of 6.49%, Ash Content of 13.88%, Nitrogen-Free Extract (NFE) of 36.20%, and Metabolizable Energy (ME) of 2952.24 kcal/kg. Additionally, BSSM contained the following anti-nutritional factors: Tannin at 2.50%, Saponin (mg/100g) at 20.50, Phytate at 0.5%, and Oxalate (mg/100g). The observed crude protein value of 30.85% for sorrel seed meal (SSM) in this study aligns with the 30.50% reported by Igoche (2015). It also agrees with the reports of Isadahomen *et al*. (2006) and Kwari *et al*. (2011) indicating sorrel seed meal (SSM) crude protein content ranging from 21.40% to 38.75%. The recorded crude fiber value of 5.28% is lower than the 11.98 and 10.20% reported by Mukhtar (2007) and Sanni (2015) respectively, but higher than the 4.18% reported by Igoche (2015). The ether extract value of 6.49% is notably lower than Mukhtar (2007) value of 17.43% and slightly higher than Sanni (2015) value of 5.78%. The metabolizable energy value of 2952.24 kcal/kg aligns with the range of 2880-3500 kcal/kg reported by various authors (Isidahomen *et al*., 2006; Mukhtar, 2007; Diarra *et al*., 2011; Kwari *et al*., 2011). Variations in these values could be attributed to differences in seed meal sources, processing methods employed, and storage conditions, as reported by Wafar (2013).

The impact of Boiled Sorrel Seed Meal (BSSM) on heamatological and biochemical indices in broiler chickens is shown in Table 4. The birds in control group (T_1) exhibited lower PCV values compared to T_2 and T_3. Additionally, Hb and RBC values were higher in T_2 and T_3 compared to T1. A linear increase in PCV, Hb, and RBC was observed with increasing BSSM inclusion, but these parameters remained non-significant across treatments, aligning with normal reference ranges for chicken heamatology (Jain, 1993). Similar findings were noted by Aliyu *et al*. (2020) and Igoche (2015), indicating no significant differences in PCV, Hb, and RBC with sorrel seed meal supplemented with protease enzymes and amino acids respectively. However, Angbulu *et al*. (2020) reported significant differences, contrasting with the results of this study. The non-significance of PCV, Hb, and RBC suggests adequate oxygen carrying capacity and absence of anaemia in the birds, potentially improved by BSSM inclusion, as indicated by Togun *et al*. (2007). Normal heamatological values indicate the safety of including BSSM in broiler diets without adverse effects (Bawala *et al*., 2007). The differential blood counts, except for lymphocytes, showed no significant differences across treatments. The elevated lymphocyte count may indicate environmental health issues rather than dietary effects, as suggested by Anon (1980). The non-significant WBC values in this study is consistent with the reports of Onunkwo *et al*. (2019) and Angbulu *et al*. (2020), although Eburuaja *et al*. (2017) reported significant differences.

Table 3. Proximate composition of raw and boiled sorrel seed meal

Nutrients	Raw sorrel seed meal	Boiled sorrel seed meal
Dry matter	93.01	92.7
Crude protein	28.44	30.85
Crude fiber	6.81	5.28
Ether Extract	5.25	6.49
Ash	12.96	13.88
Nitrogen free extract	39.55	36.20
ME (Kcal/kg)	2881.55	2952.24
Anti-Nutritional Factor concentration (g)		
Tannin (%)	4.00	2.50
Saponin (mg/100mg)	30.00	20.50
Phytate (%)	0.63	0.51
Oxalate (mg/100mg)	0.81	0.51

ME=metabolizable energy.

Table 4. Haematological and biochemical indices of broiler chickens fed graded levels of boiled sorrel seed meal.

Parameters	Treatment Levels of BSSM			SME	*p-value*
	T_1 (0 %)	T_2(10%)	T_3(20%)		
PCV (%)	19.50	24.83	25.50	1.92	0.09
Hb (g/l)	10.50	11.32	12.20	0.49	0.10
RBC($\times 10^6$/dl)	2.52	3.2	3.30	0.25	0.07
Differential counts					
Neutrophils (%)	67.33	50.33	57.33	5.13	0.09
Eosinophils (%)	1.00	1.66	1.00	1.52	0.89
Lymphocytes (%)	29.66	47.83	42.00	4.79	0.04
Serum biochemical indices					
Total protein (g/dl)	73.45	75.81	72.98	2.32	0.66
Albumin (g/dl)	44.07	45.49	43.79	1.39	0.66
Globulin (g/dl)	29.38	30.32	29.19	0.93	0.66
Triglyceride	1.78	1.91	1.75	0.08	0.33
Glucose	7.55	8.51	7.43	0.66	0.47
Alanine Transaminase	10.98^{a}	9.21^{ab}	7.55^{b}	0.74	0.01

SME=Standard Mean Error; BSSM= Boiled Sorrel Seed Meal; a, ab, b= means within the same row bearing different superscript differ significantly ($p<0.05$).

The non-significant effects of BSSM on total protein (TP), and its fractions (albumin and globulin) could be due to the protein quality in sorrel seed, supported by previous researches (Ismail *et al*., 2008; Diarra *et al*., 2011). This aligns with Onunkwu *et al*. (2019) but contrasts with Angbulu *et al*. (2020). Non-significant albumin and globulin values indicate proper clotting ability and immune function in the birds, reducing mortality (Robert *et al*., 2003; Awojobi and Opiah, 2000; Aletor *et al*., 1998). The significant alanine transaminase (ALT) values suggest liver malfunction, while non-significant triglyceride values indicate no adverse effects on heart function, consistent with Hagan *et al*. (2022). Non-significant glucose values imply increased energy in the form of glucose due to BSSM inclusion.

Conclusion and Recommendation

In conclusion, the study found that substituting soybean meal with boiled sorrel (Hibiscus sabdariffa) seed meal (BSSM) at a level of up to 20% had no detrimental effects

on the hematological and biochemical parameters of broiler birds. This suggests that adopting this replacement level of 20% BSSM could significantly alleviate the heavy reliance on soybean meal as a protein source in poultry diets. Poultry farmers are therefore encouraged to consider using BSSM as an alternative plant protein source, particularly with the boiling processing method, which has shown promising results. Furthermore, it is advisable to explore other processing methods to determine the most effective way to reduce anti-nutritional factors to their lowest levels in sorrel seed meal. This could further enhance its suitability and effectiveness as a viable substitute for soybean meal in poultry diets.

CONFLICT OF INTEREST

The authors do not have any conflicting interests.

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