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Effect of *Awara* Waste (Soybean By-Product) on Haematology and Serum Biochemical Parameters of Broiler Chickens

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ABSTRACT

This experiment was conducted to determine the effect of *Awara* waste (soybean by-product) on hematology and serum biochemical parameters of broiler chickens. Five diets were prepared as follows: Control: soybean meal-based basal diet, AW15: basal diet added with 15% *Awara* waste, AW30: basal diet added with 30% *Awara* waste, AW45: basal diet added with 45% *Awara* waste and AW60: basal diet added with 60% *Awara* waste. Two hundred 4-week-old Ross 308 broilers were randomly divided into five groups of 40 birds and each group was also divided into four replicates in a completely randomized design. The experiment lasted for 42 days, during which *ad libitum* feed and water were provided. Hematology results showed that only red blood cells and hemoglobin levels were significantly affected ($p<0.05$) by the diets. The AW30 diet showed similar RBC levels to the control on days 14 and 42, with higher levels compared to other diets. AW30 had highest Hb level on day 14, similar to the control diet on day 42. Total protein, albumin, globulin and cholesterol levels varied significantly ($p<0.05$) among diets. Control and AW30 diets had higher total protein on days 14 and 42, albumin levels were higher in the control diet on both days but lower in AW15-60, and globulin levels were higher in AW15 diet. Cholesterol levels were higher in control and AW45-60 on day 14, and lower in AW15-30. Based on these findings, AW15-30 is recommended for broiler chickens.

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INTRODUCTION

Soybeans (*Glycine max*) are a valuable source of high-quality vegetable protein, containing around 44-48% crude protein (Alada et al., 2004) with amino acids similar to meat (Tokede et al., 2015; Rizzo and Baroni, 2018). However, soybeans and other conventional protein sources are more expensive than energy sources (Ajayi, 2014), and in poultry feed, soy constitutes the bulk of the protein component (Zewdu and Berhan, 2014; Belay and Negesse, 2019). The high price and scarcity of soybeans and the fierce competition between humans and livestock seen in most developing countries have unnecessarily increased feed costs. Reducing the proportion of soy in the diet would reduce feed costs (Ajayi and Imouokhome, 2015), and this task forced poultry producers and nutritionists to look for alternative protein sources (Onunkwo et al., 2018; Ojediran et al., 2022). One of such alternative protein sources is *Awara* waste. *Awara* is the Hausa name for a snack made from soybeans and is the primary use of soybeans in northern Nigeria (Abubakar and Bello, 2017) compared to soy milk yogurts, tofu cheese and soy bread elsewhere in the world. *Awara* is made by soaking the seeds in water for about 12 hours. The water is then decanted and the seeds are ground into a paste. Water is added to the dough again and sieved (Oyeyinka et al., 2019; Adeyemo et al., 2020). The ungrounded particles left on the sieve are called waste. It contains about 25% crude protein (Sudik et al., 2020, 2023), which corresponds to 25% in cotton cake (NIAS, 2021) and more than 16.70% in palm kernel flour (Heuzé et al., 2019; NIAS, 2021). The soaking and milling processes under taken to produce *awara* will probably reduce trypsin inhibitors and other known nutritional factors in soybeans, such as lectins and allergenic proteins (Newkirk, 2010; Adeyemo et al., 2020). *Awara* is produced in both villages and towns in northern Nigeria, and large amounts of waste are dumped indiscriminately littering the environment. Dikko et al. (2018) conducted a study where they fed broiler chickens with *Awara* waste, finding that replacing soybean with 20% *Awara* was optimal and this level is low to significantly reduce the yeaning feed costs. Since *Awara* waste is readily available, further research is needed to determine its full potential in chickens' feeds. Nworgu (2004) and Obadire et al. (2019) opined that using cheaper alternative ingredients in higher quantities can significantly reduce feed costs. To ensure the birds' health is not compromised by increased levels of *Awara* waste, regular blood tests were conducted. Maxwell et al. (1990) and Togun et al. (2007) recommended thorough blood analysis to assess animals' health when introducing new diets. They emphasized that extensive blood analysis can reveal any potential health issue. The normal blood values for broiler chickens include red blood cells (2.5-4.5 x 10^{-6} μl), hemoglobin (8-14 g/dl), hematocrit (25-45%), and white blood cells (3-10 x 10^{-3} μl) (Al-Fataftah and Abdelqade, 2016). This study aimed to evacuate the effect of *Awara* waste (soybean by-product) on the hematology and serum biochemical parameters of broiler chickens.

MATERIAL and METHOD

Ethical Consideration

This study adhered to the ethical guidelines set by the Ethical Committee of the Federal University, Gashua, Yobe State, Nigeria, regarding the use of animals in biomedical research (No: 03/2023).

Table 1. Chemical analyses of Awara waste

Proximate composition	%
Moisture content	10.44
Crude protein	25.07
Ash	18.21
Ether extract	6.62
Crude fiber	7.53
Mineral composition (mg/100g)	mg/100g)
Nitrogen free extract.%	32.13
Calcium	3.17
Phosphorus	4.26
Sodium	3.84
Potassium	6.91
Magnesium	4.07
Zinc	66.94
Iron	41.63
Cobalt	6.06
Fiber fractions (%) and anti-nutritional factors	%
NDF neutral detergent fiber	56.19
ADF acid detergent fiber	40.65
ADL acid detergent lignin	2.36
Hemicellulose	16.53
Cellulose	38.29
Trypsin inhibitors (mg/kg)	2.03
Phytic acid	0.04
Essential amino acid compositions (g/16gN)	g/16gN
Arginine	3.93
Histidine	2.59
Isoleucine	3.12
Leucine	7.38
Lysine	6.34
Methionine	1.81
Phenylalanine	1.79
Threonine	2.29
Valine	4.38

Study Location

This study was conducted in the teaching and research farms of the Department of Animal Science, Federal University Gashua, Yobe State, Nigeria. Gashua is located at latitude 12°, 52.547/12.8758°N and 11.0120°/11°00.719E; and it is situated in the Sahel savanna zone of Nigeria (Ovimaps, 2014).

Nutritional Composition of *Awara* Waste

Fresh *Awara* waste was collected from production houses in Gashua City, sun-dried for five days, and analyzed in the laboratory to determine its nutritional composition, including proximate composition, mineral contents, fiber fractions, and amino acid profile. Various specific methods and instruments were utilized for each type of analysis: proximate by method described by AOAC (2005) and nitrogen free extract was by difference, fiber fractions as described by Van Soest et al. (1991), Atomic Absorption Spectrophotometer was used for minerals, phytate was determined as described by Phillipson (2000), trypsin inhibitors by Yasothai (2016), and Beckman Amino Acid Analyzer was used to determine amino acids as earlier cited by Sudik et al. (2019). The results are presented in Tables 1 after being analyzed in triplicate.

Experimental Diet

Five diets with the similar protein and calorie contents were prepared as follows: Control: soybean meal-based basal diet, AW15: basal diet added with 15% *Awara* waste, AW30: basal diet added with 30% *Awara* waste, AW45: basal diet added with 45% *Awara* waste and AW60: basal diet added with 60% *Awara* waste (Table 2).

Table 2. Ingredients and composition (%) of experimental diets used in the study

Ingredients	**Control**	**AW15**	**AW30**	**AW45**	**AW60**
Maize	52.00	51.50	51.45	51.00	51.00
Wheat offal	8.00	8.00	8.00	8.00	7.85
Awara waste	0.00	2.55	5.10	7.65	10.20
Full fat soybean	17.00	14.45	11.90	9.35	6.80
Groundnut cake	12.10	12.60	12.60	13.05	13.30
Fish meal	4.00	4.00	4.05	4.05	4.05
Bone meal	3.00	3.00	3.00	3.00	2.90
Limestone	1.00	1.00	1.00	1.00	1.00
Premix*	0.25	0.25	0.25	0.25	0.25
L-Lysine HCL	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.15	0.15	0.15	0.15
Common salt	0.25	0.25	0.25	0.25	0.25
Vegetable oil	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrients (%)					
Crude protein	20.56	20.60	20.49	20.51	20.46
Metabolizable energy (kcal/kg)	3208.50	3208.60	3208.11	3207.96	3208.01
Calcium	1.08	1.09	1.09	1.09	1.06
Phosphorus	0.51	0.51	0.51	0.50	0.50
Lysine	1.09	1.10	1.11	1.11	1.12
Methionine	0.52	0.53	0.55	0.54	0.55
Crude fiber	3.65	3.67	3.69	3.71	3.72

Control: soybean meal-based basal diet, AW15: basal diet added with 15% *Awara* waste, AW30: basal diet added with 30% *Awara* waste, AW45: basal diet added with 45% *Awara* waste and AW60: basal diet added with 60% *Awara* waste. *Vitamin premix supplied per kg diet to supply; vit A 10,000.00iu, D3,1440iu; VitE,21.6mg; VitK3,2.7mg; VitB1,1.8mg; VitB2,3.6mg: VitB6,2.7mg; Niacin,21.6mg; VitB12,0.018mg; Folic Acid,0.54mg; Panthothenicacid,9.0mg; Biotin,0.036mg; Cholinechloride,270mg; Zinc,27mg; Mn,108mg; Fe,18mg; I2, 0.72mg; Se,0.072mg; Cu,1.44mg; Co,0.144mg.

Animal Material and Management

Two hundred (200) broilers from the Ross 308 breed were obtained from the Department of Animal Science at the Federal University in Gashua, Nigeria, at 4 weeks old. The birds were randomly divided into five groups with four replicates each, with 10 birds per replicate, using a completely randomized design. They were raised in a ventilated deep litter house and given feed and water freely, with antibiotics and coccidiostats provided in the first two weeks to prevent infection. Wood shavings were used for bedding and changed weekly. The experiment lasted 42 days.

Blood Sampling and Hematological Parameters

On days 1, 14 and 42 a total of 120 blood samples (5 ml per bird) with 6 samples per replicate were randomly collected from the left-wing net. Blood for hematology was collected directly into labeled tubes containing ethylendediaminetetraacetic acid (EDTA) anticoagulant and immediately shaken to ensure proper mixing of blood and anticoagulant, while for serum biochemistry, blood was collected into labeled plain vials with no anticoagulant tip. All samples were transported to the laboratory within 30 minutes of collection in a cooler. Hematology examination was determined according to the method described by Benjamin (1998). Red cell indices such as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated using the following formulas:

MCV = PCV / RBC x 10; MCH) = Hbc / RBC x 10; MCHC = Hbc / MCH x 100.

Randox Diagnostic Kit was utilized to measure total blood protein, creatinine, and serum albumin levels as adopted by Sudik et al. (2019). Globulin level was calculated by subtracting albumin from total protein as described by Omodanisi et al. (2017). Alanine aminotransferase and aspartate aminotransferase activities in plasma were tested (Varley et al., 1980), and cholesterol levels were determined following the procedure outlined by Wieland and Seidel (1983).

Statistical Analysis

Data were analyzed in one-way analysis of variance (ANOVA) using SPSS statistics 25 for Windows program (IBM, version 25, Chicago, USA). Significant differences among treatment means were determined using Duncan's multiple range test (DMRT). Significant difference was based on $P < 0.05$.

The following completely randomized design model was used to evaluate the production function:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = individual observation

μ = Overall mean

Ti = Treatment effect (i=1, 2, 3, 4, 5)

e_{ij} = Random error

RESULTS and DISCUSSION

Table 3 compares the haematological parameters of broiler chickens fed diets containing different levels of *Awara* waste. The significant differences were seen in red blood cell count (RBC) and hemoglobin (Hb) levels. Birds fed the AW30 diet had similar RBC levels to the control on days 14 and 42, and higher levels compared to other diets. The Hb levels followed a similar trend, with AW30 showing the highest levels on day 14 and similar levels to the control on day 42. The rise in RBC and Hb with increasing AW levels up to 30% in the diet indicates improved oxygen transport to the cells, supporting the birds' metabolic rate and overall health. The results contrast with the findings of Dikko et al. (2018), where they noted significant different in white blood cells (WBC). The discrepancies between the studies could be attributed to environmental and management factors as discussed by Brown et al. (2019) and Smith et al. (2020). The absence of significant differences in white blood cell counts suggests no infections from consuming AW. Overall, all blood parameters remained within normal ranges (Merck, 2012) which indicates that incorporating AW did not compromise the birds' nutritional requirements and well-being. This supports the notion that a balanced diet is crucial for maintaining normal blood counts and disease resistance in poultry (Leeson and Summers, 2005). Additionally, the NRC (1994) stresses that a diet lacking essential nutrients like iron and copper can lead to deficiencies and health problems like anemia in poultry.

Table 4 displays the serum biochemical parameters of broiler chickens fed diets with different levels of *Awara* waste. The results showed significant differences ($p<0.05$) in total protein, albumin, globulin, and cholesterol levels among the diets. Control and AW30 diets had higher total protein levels on days 14 and 42, suggesting the quality protein content in AW30 was similar to the control. Albumin levels were higher in the control group on both days but lower in AW15-60. Globulin levels were higher in AW15 on both days and lower in other diets. Cholesterol levels were higher in control, AW45, and AW60, but lower in AW15 and AW30 on day 14. Dikko et al. (2018) also reported significant different in albumin and LD cholesterol. Lower cholesterol levels in AW15 and AW30 may be due to their lower contents of both full fat soybean and groundnut cake (Table 2).

Table 3. Hematological parameters of broilers fed diets containing different levels of *Awara* waste

Parameters	Ages	Control	AW15	AW30	AW45	AW60	SEM	**p-value**
RBC x106 µl	1 d	3.12	3.11	3.12	3.12	3.11	0.01	0.790
	14 d	4.06[a]	3.63[b]	3.91[a]	3.70[b]	3.61[b]	1.26	0.000
	42 d	4.23[a]	3.92b	4.21a	4.00[ab]	3.93b	0.15	0.000
PCV (%)	1 d	29.24	29.21	29.18	29.37	29.22	0.07	0.328
	14 d	30.17	29.91	30.45	30.16	29.67	0.29	0.100
	42 d	31.07	31.12	31.35	30.78	30.28	0.41	0.100
Hb (g/dl)	1 d	11.44	1135	11.68	11.26	11.55	0.17	0.233
	14 d	12.26[b]	11.13[c]	13.01[a]	11.06[c]	11.11[c]	0.88	0.000
	42 d	13.23[a]	11.87[b]	13.26[a]	12.07[b]	12.09[b]	0.68	0.000
WBC x 103 µl	1 d	3.10	3.00	3.09	3.10	3.11	0.08	0.178
	14 d	3.11	3.10	3.14	3.12	3.15	0.02	0.201
	42 d	3.21	3.09	3.23	3.45	3.35	0.14	0.311
Lymphocytes	1 d	62.16	62.18	61.13	62.16	62.53	0.17	0.588
	14 d	63.11	62.48	63.13	63.01	62.47	0.34	0.571
	42 d	63.98	63.18	64.70	63.10	62.72	0.79	0.411
Neutrophils	1 d	22.45	22.45	22.45	22.45	22.37	0.04	0.100
	14 d	21.12	22.04	21.25	21.24	22.41	0.58	0.135
	42 d	21.01	21.65	20.17	21.55	22.20	0.77	0.147
Monocytes	1 d	12.12	12.15	12.19	12.2	12.01	0.08	0.276
	14 d	12.40	12.25	12.3	12.4	12.01	0.16	0.201
	42 d	12.00	12.13	12.1	12.13	12.01	0.06	0.110
Eosinophils	1 d	2.27	2.22	2.23	2.19	2.09	0.07	0.378
	14 d	2.37	2.23	2.32	2.35	2.11	0.11	0.790
	42 d	2.03	2.04	2.03	2.22	2.07	0.08	0.791
Basophils	1 d	1.00	1.00	1.00	1.00	1.00	0.00	0.100
	14 d	1.00	1.00	1.00	1.00	1.00	0.00	0.100
	42 d	1.00	1.00	1.00	1.00	1.00	0.00	0.100
MCV	1 d	80.02	80.34	80.39	80.62	80.28	0.22	0.288
	14 d	81.21	81.55	82.17	81.75	79.31	1.10	0.218
	42 d	82.22	82.16	84.83	82.29	81.10	1.38	0.136
MCH	1 d	26.11	26.67	27.33	26.16	27.13	0.55	0.168
	14 d	27.45	27.14	28.21	26.11	26.40	0.84	0.145
	42 d	27.48	27.86	28.35	28.38	27.37	0.47	0.217
MCHC	1 d	31.04	31.20	31.16	31.40	31.18	0.13	0.178
	14 d	31.11	31.36	31.47	31.61	31.40	0.18	0.101
	42 d	21.62	21.15	21.38	21.16	21.37	0.19	0.166

Control: soybean meal-based basal diet, AW15: basal diet added with 15% *Awara* waste, AW30: basal diet added with 30% *Awara* waste, AW45: basal diet added with 45% *Awara* waste and AW60: basal diet added with 60% *Awara* waste, Values are mean of 24 birds, RBC = red blood cells, WBC = white blood cells, PCV = Packed cell volume, Hb = haemoglobin, MCV = Mean cell volume, MCH = Mean cell haemoglobin and MCHC = Mean cell haemoglobin concentration. Means in the same row not sharing common superscript are significantly different (p<0.05).

According to NIAS report (2021), full fat soybean contains more fat than its byproducts. These fluctuations in serum protein, albumin, globulin, and cholesterol levels, though they fell within normal ranges: 2.5-4.5, 1.5-3.5, 0.5-2.5 and 100-300 mg/dl, respectively (Merck, 2012; Smith et al., 2018) indicate proper immune system functioning. In essence, globulins are a collection of proteins that play a role in fighting infections and supporting bodily functions, while cholesterol is a crucial type of fat for cell and hormone functioning (Campbell, 1995; Ritchie et al., 1994).

Table 4. Serum biochemical parameters of broilers fed diets containing different levels of *Awara* waste

Parameters	Ages	**Control**	**AW15**	**AW30**	**AW45**	**AW60**	**SEM**	***p*-value**
	1 d	3.75	3.71	3.82	3.74	3.71	0.05	0.204
Total protein (mg/dl)	14 d	4.01^{a}	3.91^{b}	4.11^{a}	3.79^{c}	3.73^{c}	0.16	0.011
	42 d	4.44^{a}	4.00^{b}	4.37^{a}	3.88^{c}	3.87^{c}	0.27	0.001
	1 d	2.26	2.22	2.27	2.18	2.12	0.06	0.317
Albumin (mg/dl)	14 d	2.56^{a}	2.29^{b}	2.43^{b}	2.29^{b}	2.21^{b}	0.14	0.016
	42 d	2.66^{a}	2.33^{b}	2.63^{b}	2.35^{b}	2.33^{b}	0.17	0.01
	1 d	1.49	1.49	1.55	1.56	1.59	0.04	0.505
Globulin (mg/dl)	14 d	1.45^{d}	1.82^{a}	1.68^{b}	1.60^{b}	1.52^{c}	0.09	0.006
	42 d	1.78^{b}	1.87^{a}	1.74^{b}	1.53^{c}	1.54^{c}	0.11	0.125
	1 d	60.11	60.22	60.21	60.47	60.12	0.15	0.221
AST (U/L)	14 d	61.31	61.13	60.27	6154	62.36	0.75	0.113
	42 d	59.51	59.48	58.19	61.38	62.32	1.65	0.353
	1 d	25.11	26.23	25.29	27.42	27.31	1.09	0.405
ALT (U/L)	14 d	26.57	26.45	26.51	29.12	31.14	2.11	0.416
	42 d	30.8	29.32	26.26	28.27	30.24	1.80	0.115
	1 d	115.23	125.45	125.23	135.21	125.13	7.06	0.611
Cholesterol (mg/dl)	14 d	121.66^{a}	126.15^{b}	116.57^{b}	126.34^{a}	122.16^{a}	4.00	0.001
	42 d	130.14	120.11	130.6	131.3	129.41	4.64	0.038
	1 d	12.16	12.73	11.74	12.65	11.74	0.48	0.25
Uric acid (mg/dl)	14 d	11.44	12.26	12.18	11.91	11.72	0.34	0.309
	42 d	11.18	12.33	11.55	12.37	12.23	0.54	0.25
	1 d	1.74	1.64	1.24	1.28	1.24	0.24	0.45
Creatinine (mg/dl)	14 d	0.81	1.08	1.06	0.99	1.06	0.11	0.163
	42 d	0.77	0.55	1.01	1.10	1.06	0.23	0.39

Control: soybean meal-based basal diet, AW15: basal diet added with 15% *Awara* waste, AW30: basal diet added with 30% *Awara* waste, AW45: basal diet added with 45% *Awara* waste and AW60: basal diet added with 60% *Awara* waste, Values are mean of 24 birds, AST =aspartate amino transferase, ALT = alanine amino transferase. Means in the same row not sharing common superscript are significantly different ($p<0.05$).

This research suggests that using *Awara* waste in broiler chickens' diets did not harm their immune systems, livers, kidneys, or muscles, as there were no noteworthy differences in key parameters such as liver enzymes, uric acid and creatinine. Normalization of liver enzymes, uric acid, and creatinine levels post-diet indicate the safety of the test ingredient (Campbell et al., 2013; Jain, 1986), while abnormal levels may indicate liver, renal, or muscle damage (Campbell, 2007; Schmidt et al., 2007; Traesel et al., 2011).

CONCLUSION

The research indicated that adding *Awara* waste to broiler chickens' diet did not have a negative impact on their immune system, livers, kidneys, and muscles, as there were no significant differences in various parameters such as white blood cells, liver enzymes, uric acid, and creatinine. In fact, incorporating *Awara* waste at 15-30% levels was found to enhance red blood cell production, increase serum protein and globulin levels, and reduce cholesterol levels. Thus, replacing soybean meal with *Awara* waste in broiler chickens' diets at these levels is recommended for their overall health and well-being.

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Authors' Contribution

Conceptualization, SSD; methodology and investigation, MOJ; investigation, SSD, MOJ; data curation, SSD, MA; writing original draft preparation, SSD; writing, review and editing, SSD, MOJ, LT, MA; project administration, SSD, MOJ, LT, MU. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

We declare that there are no conflicts of interest.

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