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Effects of equi-protein replacement of soybean meal with graded levels of differently processed rubber seed meal on health status of broiler chickens

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Abstract

The nutritional qualities of feed stuff are best examined on the effect of it on the health status of animals, this experiment was conducted to evaluate the effect of incorporation of graded levels of differently processed rubber seed meal (RSM) into broiler chicken diets on the health status of the birds. Two samples of RSM were subjected to different processing methods; soaking in hot water (HWRSM) and roasting (RRSM) and were incorporated into broiler chicken diets to replace soya bean meal at 15, 25 and 35% equi-protein replacement levels in six weeks feeding trial. At maturity of the birds, blood was collected for haematological (packed cell volume; erythrocyte sedimentation rate; Red blood cells; Haemoglobin concentration; mean cell volume; mean cell haemoglobin and mean cell haemoglobin concentration; lymphocytes; neutrophils; monocytes; basophiles and eosinophil) and Serological parameters (total serum protein; aspartic aminotransferase; alkaline aminotransferase; cholesterol; urea; albumin; globulin; albumin / globulin and creatinine). The result showed that there were no significant ($P \geq 0.05$) differences in all haematological variables and serological indices measured except cholesterol, as compared with those birds on soybean meal-based diets. The values of cholesterol of birds on the dietary treatments were significantly lower than those in soybean meal-based diet. The average values of all haematological variables; packed cell volume (27.50 - 28.67%); erythrocyte sedimentation rate(3.00 -3.33%); Red blood cells (3.10 - 3.70%); Haemoglobin concentration (9.15 - 9.55%); mean cell volume (81.70- 93.07%); mean cell haemoglobin (27.31-31.01pg) and mean cell haemoglobin concentration (33.16-33.37 g/dL) lymphocytes (30.17-31.33%); neutrophils (22.17- 24.50%); monocytes (10.50 -13.33%); basophiles (2.33 -2.83%); eosinophil (0.67 -1.33%) and serological indices; TSP (3.16 -3.74 mg/dl); aspartic aminotransferase (16.50 - 21.30 µ/d); alkaline aminotransferase (4.84 -10.40 µ/d), cholesterol (107.64 -146.32 mg/dL); Urea (10.22 - 11.08 mg/dL); Albumin (11.63 - 14.18 g/dL) Globulin (7.47 -17.70 g/dL); Albumin / globulin (0.85 - 2.79) and creatinine (4.98-6.80 mg/dL) were within the established range for healthy birds. Suggesting that feeding of processed rubber seed meals-based diets have no noticeable adverse effect on health status of broiler chickens and can be included in their meal at these levels.

Keywords: Equi-protein; rubber seed meal; soybean meal; haematology; serology

Introduction

Poultry production is the most economic route for producing high quality animal protein within the shortest possible time for the rapidly increasing human population across the world because of its short gestation period, no need of storage being a meal size and for the fact its consumption is widely accepted. However, feeding has become a serious limiting factor. Adeyemi et al. (2008) reported that feeding accounted for about 60-70% of total cost of production of livestock under intensive management in Nigeria. The economics of Poultry feeding are largely dependent on the local condition of feedstuff availability and competition for the same feedstuff for

use by human. In a bid to combat or reduce the competition between man and animals for the same feed resources, minimize the cost of poultry production, increase availability of feed ingredients and maximize profitability of livestock business by farmers, the nutritional potentials of unconventional feed ingredients which can either be from plants or animal origin, need to be investigated and utilized by animal nutritionists. Research has shown that the nutrient composition of rubber seed meal (RSM) were; metabolizable energy (1828.65- 2675.61kcal/kg), crude protein (22.16- 40.36 %,), ether extract (12.41 to 55.67%), crude fibre (2.65 - 3.62 %,), ash (2.20%- 6.57%) and carbohydrate (14.09 - 41.48%) (Oyewusi et al., 2007, Eka et al., 2010 and Akinsanmi et al., 2017). Madubuike et al. (2006) reported that Nigeria rubber seed is high in oil content (49.49%), phosphorus (0.82%) and rich in essential amino acid like L-lysine (3.60%) and methionine (1.4%). The range of metabolic energy of RSM (1828.65-2675.61kcal/kg) falls within the range of metabolizable energy (ME) in other pulses used in feeding livestock. Olomu (2011) reported the values of 2750, 2460 and 2069 ME (Kcal/kg) for groundnut cake, soybean cake and cotton seed cake respectively. The seed meal or cake of rubber have been reported to have higher contents of digestible nutrients than some conventional seed meals and are highly promising as future protein supplements in livestock and animal diets (Oyewusi et al., 2007). Though RSM is reported to be high in cyanide (18mg/100g) by Eka et al. (2010), Akinsanmi et al. (2017) confirmed that those anti nutritional factors found in rubber seed are heat labile that can be reduced to tolerable level that will have adverse effect on the performance of the animal through processing. Examination of blood is a good way of assessing the health status of an animal, it gives room for clinical investigation of the presence of several metabolites and other constituents in the body of animals and also plays vital role in the physiological, nutritional and pathological status of an organism (Onifade, 1993). Haematological parameters provide valuable information on the immune status of animals which is a good source of information for diagnostic and management purposes and could equally be incorporated into breeding programs for the genetic improvement of poultry (Elagib and Ahmed, 2011). Haematological and serum biochemical changes have also been used to evaluate the level of stress due to environment or nutritional factors (Mmereole, 2004). In addition, haematological parameters have been found to be associated with certain production traits in chicken. For example, high packed cell volume and high haemoglobin are indicators of high feed conversion efficiency while high serum protein is an indicator of good feathering ability and tissue growth (Ojebiyi et al., 2009). Mmereole (2009) reported that the blood profiles are a base reference for overall production management purposes, the breed or strain and the age of the birds should be taken into consideration in arriving at any decision. Haematological and serum biochemical analyses have been used as a tool in the diagnosis of many diseases, in evaluating the responses to therapy in both animals and human beings (Ameen et al., 2007) Haematological studies entails the assessment of various blood parameters such as total red blood cell counts (RBC) haemoglobin count (HB), packed cell volume (PCV), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR) and coagulation time. Other blood

indices that can be assessed include total white blood cell counts (WBC) and differential leukocyte counts. It has also been observed that as birds get older, the values for WBC, RBC, HB, and PCV increased while the values for MCV, MCH, and MCHC decreased (Ojebiyi *et al.*, 2009). Haematological indices of chickens are affected by a number of factors such as age, sex, hormones, oxygen level, medications, stress, breeds, nutrition and diseases (Ojebiyi *et al.*, 2009). Aletor and Ebgerongbe (1992) reported that changes at haematological levels usually precede the onset of outward signs as weight changes, loss of hair or feathers, changes in milk or egg production following dietary treatment. Thus, blood constituents and its functions are reported to be subjected to a large measure of dietary influence such as the quantity and quality of protein, vitamins and minerals as well as the presence of anti-nutrients (Ojebiyi et al., 2009) and this form the basis for this research.

Methodology

This experiment was carried out at the Teaching and Research Farm of the Federal University of Technology; Akure which is located between 7.15^{0} North and 5.0^{0} East of the equator with the average annual rainfall of 1524mm and annual temperature of 28^{0c} to31^{0c} and mean annual relative humidity of about 80% (Ajibefun, 2011). The rubber seeds used for this study were purchased from Ilusin rubber estate, Ilusin, Ogun state, Nigeria. The seeds were washed with distilled water, sundried, dehulled and allowed to pass through two different processing methods: (a) soaking in hot water for 12 hours (HWRSM) and (b) roasting for 15 minutes and cooled at room temperature (RRSM). The samples were dried, milled and chemically analysed. Proximate analysis of the samples was carried out using the AOAC (1995) method. Based on the result of the chemical compositions (Akinsanmi et al., 2017), the two samples with best chemical compositions (RRSM and HWRSM) were selected for the experiment. Two hundred and fifty day-old broiler chicks of Hubbard strain were purchased from AFGRI Bnot Harel hatchery, Ibadan, Oyo-State, Nigeria, out of which 210 were selected for the experiment. The chicks were weighed and randomly allotted to seven dietary treatments, 30 chicks /treatment and 10 chicks/ replicate. The experimental design was completely randomized design with 2 x 4 factorial arrangement (two processing methods; roasting and soaking in hot water and four levels of equi-protein replacement of soybean meal (0, 15, 25 and 35%). The chicks were fed with their dietary diet and water was supplied ad-libitum for six weeks. At the 42^{nd} day of the fattening period 12 birds per treatment were randomly selected for carcass evaluation, making a total of 84 birds. They were weighed and slaughtered. After slaughtering, the birds were bled and the blood collected. Blood samples for haematological parameters were collected into tubes containing ethylene diamine tetra acetic acid (EDTA) to prevent coagulation. Blood samples for serum parameters were collected in separate tubes without EDTA. The blood was allowed to coagulate and after six hours the serum was separated into sterile universal bottles and kept deep-frozen (-20^{O}C) prior to analysis. The haematological parameters determined were red blood cell (RBC), pack cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). Other parameters include lymphocytes, neutrophils, basophils, monocytes and eosinophil. The haematological parameters were determined by the use of improved

Neubauer heamocytometer (Lamb, 1991). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were computed using the formulae outlined by Jain (1986). Samples for biochemical parameters were first centrifuged and decanted. Sigma kits were thereafter used to determine protein, globulins, albumins, urea, creatinine, cholesterol, calcium, phosphorus. Serum aspartic aminotransferase (AST), alkaline aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using the procedure of Baker and Silverton (1985).

Table 1: Gross composition (%) of broiler chicken diets with graded levels of differently processed rubber seed meals

Ingredient	Control	RRSM			HWRSM		
Inclusion level	**0%**	**15%**	**25%**	**35%**	**15%**	**25%**	**35%**
Maize	53.00	50.90	49.51	48.11	49.73	47.56	45.38
Wheat offal	7.00	7.00	7.00	7.00	7.00	7.00	5.00
Soybean meal	28.00	23.80	21.00	18.20	23.80	21.00	18.20
RRSM (28.02% CP)	0.00	6.30	10.51	14.69	0.00	0.00	0.00
HWRSM (23.63% CP)	0.00	0.00	0.00	0.00	7.47	12.44	12
Groundnut cake	0.00	0.00	0.00	0.00	2.00	2.00	2.00
Fishmeal	4.00	4.00	4.00	4.00	4.00	4.00	4.00
DCP	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vegetable oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values							
ME (Kcal/kg)	3126.90	3092.00	3068.80	3045.53	3116.78	3019.45	3067.56
Crude Protein	20.43	20.15	20.13	20.09	20.62	20.42	20.23
Crude Fibre	3.52	3.40	3.32	3.25	3.34	3.28	3.21
Calcium	1.13	1.13	1.12	1.12	1.13	1.13	1.13
Av. Phosphorus	0.73	0.71	0.69	0.67	0.69	0.67	0.65
Lysine	1.38	1.58	1.72	1.86	1.66	1.84	2.01
Methionine	0.55	0.67	0.74	0.82	0.69	0.79	0.88
Analyzed values							
MC	9.00	8.44	9.50	9.00	7.91	7.93	7.93
Total Ash	9.56	9.97	9.23	9.99	8.56	9.27	8.95
Crude Protein	21.00	20.89	20.86	21.02	21.08	20.88	20.55
Crude Fibre	3.18	3.52	3.10	3.02	3.52	3.48	3.67
Fat	5.18	5.00	5.22	5.70	4.20	4.39	4.53
CHO	57.01	56.97	58.25	58.11	59.54	58.49	59.46

Keys: HWRSM; Hot water rubber seed meal , RRSM; Roasted rubber seed meal, Premix contained the following: (Univit. 15 Roche) 1500 I.U., Vit. A, 1500 I.U., Vit. D: 3000 I.U., Vit.E, 3.0 g; Vit, B2, 0.3 g; Vit. B6, 8.0 mg; Vit. B12, 8.0 g; Nicotinic acid, 3.0 g; Ca-Pantothenate; 5.0 mg; Fe, 10.00 g; Al, 0.2 g; Cu, 3.5 mg; Zn, 0.15 mg; I, 0.02 g; Co, 0.01 g/kg.

Result and discussion

Haematological and serum biochemical changes have been the parameters used to evaluate the level of stress due to environment or nutritional factors in animals (Mmereole, 2004). For example, high packed cell volume (PCV) and high haemoglobin concentration are indicators of high feed conversion efficiency while high serum protein is an indicator of good feathering ability and tissue growth. The result of this study revealed that there were no significant differences in all haematological parameters measured. The range of all haematological variables fall within their normal ranges for healthy broiler chickens. Packed cell volume (27.50-28.67%); red blood cells (3.10-3.70x10^6/mm^3); Haemoglobin (9.15-9.55g/dL) erythrocytes sedimentation rate (3.30-3.339mm/hr); mean cell volume (81.70-93.07fl); mean cell haemoglobin (27.31-31.01pg) and mean cell haemoglobin concentration (33.16-33.37g/L). Heterophils (22.17-24.50%), lymphocytes (30.17-31.33%), basophils (2.33-2.83%) and eosinophil (0.67-1.33%) were within the documented range of values for broiler chickens as reported by Ameen *et al.*(2007); Banerjee (2008) and Ladokun *et al*. (2008). It could be deduced from the range of RBC values of the birds in the present study that the experimental diets met the protein requirement of the birds. Bahman (2011) reported that the use of blood analysis is the fast means of assessing clinical and nutritional health status of animal on feeding trials.

The low ESR recorded from this study suggest an increase in total plasma protein or glucose in the birds and also indicates good heath (Ladokun *et al*., 2008). MCV which is another Haematological variable that can be used to determine the type of anaemia in an animal. It is an expression of the average volume of red blood cells in the blood. When it is high it may indicate anaemia due to nutritional deficiencies, bone marrow abnormalities, liver disease, chronic lung disease or therapy with certain medications and low MCV may indicate iron deficiency, chronic disease, pregnancy, haemoglobin disorder such as thalassaemia, anaemia due to blood cell destruction or bone marrow disorder (Ameen *et al.,* 2007). MCV values were within the range for normal reported for broiler chickens by Banerjee (2008). Togun *et al.* (2007) reported that when the haematological values fall within the normal range reported for the animal, it is an indication that the diets have no any adverse effects on the health of the animal. Therefore, all haematological variables obtained from the birds used in this experiment indicated that the birds did not suffer any blood related illness during experiment. Treatment interactions were not significantly affected so also levels interactions. This is a good reason to establish the fact that incorporation of hot water soaked rubber seed meal or roasted rubber seed meal up to 35% equi-protein replacement level of soybean meal into broiler chickens diet will not pose any health hazard to the birds. Serum chemistry is routinely used for the detection of organ disease in domestic animals. For example, the normal ranges of serum total protein values for most avian fall between 3 and 6 g/dL. Less than 3 indicate hypoalbuminca and when it is 2.5g/dL or less indicates prognosis (McDonald, 1996). Higher value than 6g/dL occurs due to dehydration or an increase in total globulins (Campbell and Coles, 1986). All serum parameters measured in this study were within the documented ranges for healthy birds. Treatment and levels did not also significantly affect serological indices except cholesterol. Cholesterol is important to the body as a constituent of cell

Table 2: Haematological variables of broiler chickens fed differently processed RSM based diets

Diets	Level	ESR	PCV	RBC	HB	LYM	NEU	MON	BAS	EQS	MCV	MCH	MCHC
Level	0	3.17	28.00	3.31	9.33	31.33	22.67	12.67	2.50	0.83	87.01	29.01	33.16
	15	3.08	28.25	3.58	9.41	31.00	23.67	11.83	2.58	0.92	83.98	28.81	33.32
	25	3.17	27.50	3.16	9.16	30.33	23.50	12.58	2.58	1.00	89.27	28.56	33.30
	35	3.33	28.08	3.37	9.36	30.75	23.67	12.08	2.33	1.17	87.39	29.82	33.34
	SEM	0.45	0.95	0.29	0.31	0.31	0.68	0..72	0.18	0.19	4.41	1.63	0.07
	P value	0.93	0.84	0.58	0.83	0.29	0.97	0.68	0.46	0.49	0.71	0.86	0.91
Treatment													
RRSM		3.22	28.17	3.40	9.39	30.78	24.33	11.50	2.39	1.00	85.32	29.47	33.34
HWRSM		3.17	27.72	3.34	9.23	30.61	22.89	12.83	2.61	1.06	88.44	28.65	33.06
SEM		0.42	0.89	0.27	0.29	0.27	0.58	0.65	0.16	0.18	3.86	1.42	0.09
P value		0.92	0.63	0.86	0.65	0.63	0.06	0.07	0.25	0.75	0.55	0.68	0.67
Treatment x	Level												
Control	0	3.17	28.00	3.31	9.33	31.33	22.60	12.60	2.50	0.83	87.01	29.01	33.16
RRSM	15	3.00	28.17	3.70	9.37	31.17	22.17	13.17	2.83	0.67	83.14	27.64	33.28
RRSM	25	3.17	27.50	3.22	9.15	30.17	22.50	13.33	2.67	1.33	89.11	27.31	33.27
RRSM	35	3.33	27.50	3.12	9.17	30.50	24.00	12.00	2.33	1.17	93.07	31.01	33.37
HWRSM	15	3.17	28.33	3.47	9.45	30.83	25.17	10.50	2.33	1.17	84.82	29.99	33.37
HWRSM	25	3.17	27..50	3.10	9.17	30.50	24.50	11.83	2.50	0.67	89.43	29.81	33.33
HWRSM	35	3.33	28.67	3.64	9.55	31.00	23.33	12.17	2.33	1.17	81.70	28.63	33.32
SEM		0.61	1.24	0.38	0.41	0.41	0.83	0.86	0.23	0.21	5.99	2.25	0.07
P value		0.98	0.89	0.60	0.90	0.58	0.10	0.28	0.53	0.06	0.54	0.53	0.72
Significance													
Level		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Treatment		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Treatment x	Level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Keys: RRSM: Roasted rubber seed meal, HWRSM: Hot water rubber seed meal, SEM: standard error of mean, PCV: packed cell volume (%), RBC: red blood cells (%), WBC: white blood cell,Hbc: haemoglobin concentration (%) MCV: mean cell volume (%), MCH: mean cellhaemoglobin (pg), MCHC: mean cell haemoglobin concentration (g/dl), lym: lymphocytes (%), Neu: neutrophils (%), Mon: monocytes (%), Eos :eosinophil (%).Bas: Basophils (%). Values are means for 30 birds /diet, NS: Not Significant ($P \geq 0.05$).

Table 3: Serological metabolites of broiler chickens fed differently processed RSM based diets.

Treatment	Level	AST (μ/dl)	ALT (μ/dl)	CHOL. (mg/dl)	UREA (mg/dl)	TSP (mg/dl)	CREA (mg/dl)	ALB (g/dl)	GLO (g/dl)	ALB/ GLO
Level	0	213.00	7.93	146.32[a]	10.38	3.42	0.68	1.37	2.46	0.56
	15	185.45	5.02	117.82[b]	10.04	3.43	0.58	1.29	2.26	0.59
	25	193.65	6.27	109.94[c]	11.22	3.74	0.63	1.39	2.36	0.59
	35	198.37	9.31	107.64[c]	10.92	3.46	0.63	1.39	2.52	0.55
SEM		0.40	1.00	9.21	1.59	0.04	0.00	0.03	0.02	0.00
P value		0.56	0.14	0.04	0.07	0.06	0.66	0.77	0.13	0.06
Treatment										
Control		213.00	7.93	146.32[a]	10.38	3.42	0.68	1.37	2.46	0.56
RRSM		191.28	6.73	109.10[b]	10.56	3.28	0.63	1.33	2.33	0.57
HWRSM		194.28	6.75	109.47[b]	10.66	3.78	0.55	1.37	2.38	0.58
SEM		8.90	1.66	8.35	1.46	0.03	0.00	0.02	0.03	0.00
P value		0.66	0.86	0.54	0.08	0.07	0.12	0.85	0.05	0.05
Treatment x	Level									
Control	0	213.00	7.93	146.32	10.38	3.43	0.68	1.37	2.46	0.56
RRSM	15	1.66.50	4.84	109.35	10.71	3.16	0.062	1.42	2.07	0.69
RRSM	25	198.60	6.96	103.99	10.37	3.67	0.66	1.24	2.24	0.55
RRSM	35	205.83	8.44	115.46	10.33	3.65	0.64	1.36	2.40	0.57
HWRSM	15	204.40	5.20	106.30	10.16	3.71	0.56	1.04	2.37	0.44
HWRSM	25	188.67	5.57	110.92	10.95	3.87	0.49	1.42	2.47	0.57
HWRSM	35	190.90	10.40	113.92	11.08	3.25	0.63	1.39	2.72	0.51
SEM		10.98	1.98	12.26	2.03	0.06	0.00	0.02	0.03	0.00
P value		0.07	0.07	0.07	0.08	0.09	0.09	0.08	0.06	0.12
Significance										
Level		NS	NS	*	NS	NS	NS	NS	NS	NS
Treatment		NS	NS	NS	NS	NS	NS	NS	NS	NS
Treatment x	Level	NS	NS	NS	NS	NS	NS	NS	NS	NS

Keys: RRSM roasted rubber seed meal, HWRSM: hot water rubber seed meal, TSP: total serum protein, ALT: alkaline aminotransferase, AST: aspartic aminotransferase, ALP: alkaline phosphatase. Cho l: cholesterol; Creat: creatinine; ALB: albumin; GLO: globulin , SEM: standard error of mean. Values are means for 30 birds /diet NS: Not Significant, * Significant. Means on the same row with different superscripts are significantly ($P \leq 0.05$) diffe. .

membranes, and involved in the formation of bile acid and some hormones (Microsoft Encarta, 2009). The high ether extract recorded for RSM in this study did not result to high serum cholesterol since birds on control diet recorded the highest cholesterol level (146.32mg/dL). This finding agreed with Enig (2000) that fat content of food is not necessarily a good indicator of its cholesterol content since meats, liver and some organs are low in fat, but very high in cholesterol. Continuous deposition of cholesterol may lead to narrowing of the coronary arteries which can lead to cardiac arrest or heart attack (Alabi, 2012). It is therefore recommended that serum cholesterol in broiler chicken should be between 74.50 to 181.50mg/dL (Bahman *et al*., 2011). Lower serum cholesterol of all RSM based diets over soybean meal based diet in this study further confirm RSM as good alternative feedstuff using the above adopted processing methods.

Conclusion

The study showed that clinical examination of blood of broiler chickens used for this experiment revealed similar responses which suggest that soybean meal and rubber seed meal have similar effects on the health status of broiler chickens. The range of values of all haematological variables measured and serological indices were

within the reported ranges for healthy birds which established the fact that incorporation of well processed rubber seed meal into poultry diet have no adverse effect on the health of animal and expand the scope of livestock feedstuffs.

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