EFFECTS OF HOUSEFLY LARVAE MEAL ON BLOOD CELLS, SERUM ELECTROLYTES AND BIOCHEMICAL PARAMETERS OF GROWER PHASE INDIGENOUS COASTAL SAVANNA ECOTYPE CHICKEN IN SOUTHERN GHANA

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ABSTRACT
Replacements of fishmeal with housefly larvae meal by 0, 25, 50, 75 and 100% in the diet of the indigenous chicken (Coastal Savanna Ecotype) in southern Ghana showed no significant difference on red blood cells, haemoglobin, packed cell volume, white blood cells, monocytes, basophils, lymphocytes, MCH and MCV. HFLM replacement also did not affect total cholesterol, triglycerides, uric acid, alanine transaminase, aspartate transaminase, direct bilirubin, total bilirubin and albumin levels. However, creatinine, alanine phosphate and total protein levels differed significantly ($p < 0.05$). Serum electrolytes, sodium ions (Na^+), potassium ions (K^+) and chloride ions (Cl^-) did not differ significantly ($p > 0.05$). Parameters showing some differences in mean values however were within acceptable levels for Coastal Savanna Ecotype. HFLM could in effect replace fishmeal as a substitute or in combination with fishmeal in poultry feed and this poses no health threats to liver, kidney and physiological ionic balance.

Keywords: *Fishmeal, housefly larvae meal, replacement, Fulani ecotype, creatinine, electrolytes,*

INTRODUCTION
A major hurdle militating against commercial poultry production is the high cost of feed ingredients especially the protein sources such as soybean cake, groundnut cake and fishmeal (Adeniji, 2007). However, there are many cheap sources of proteins which are not conducive for human consumption but are ready source for inclusion in feed formulation. There are also some animal or abattoir by-products and agriculture related by-products which are definitely of cheaper cost when used to feed monogastric animals such as chicken (Omole and Tewe, 1985). Various studies undertaken by Waldroup and Harms (1963), Calvert *et al*., (1969), Teotia and Miller (1974), Calvert (1979), Ocio and Vinaras, (1979) showed that maggots or housefly larvae can be produced from animal waste such as poultry manure and municipal organic waste resulting in a product rich in animal protein albeit limiting in some vital amino acids such as lysine, arginine and methionine. The quality of housefly larvae meal (HFLM) is reported to be comparable to that of meat or fish meal and soybean meal (Dankwa *et al*., 2002)

In this study, the effect of 0, 25, 50, 75 and 100% HFLM replacements of fishmeal on blood, serum electrolytes, serum protein and products as indicators of health and safety of HFLM in poultry feed formulations on the Coastal Savanna ecotype chicken in Southern Ghana were studied.

MATERIALS AND METHODS

Study Site and condition

This study took place at CSIR-Animal Research Institute Stations at Frafraha and Pokuasi in the Greater Accra Region of Ghana. Housefly larvae (HFL) was produced at Frafraha whilst feeding trials were done at Pokuasi. The two Stations fall under two climatic zones; Pokuasi, falls under the transition zone between the coastal savanna and the rain forest. Frafraha has a mean annual rainfall of 730mm and mean monthly temperature range of between 24.7^{o}C in August and 28^{o}Ċ . which usually occurs in March. Relative humidity is usually high ranging from 65 to 95% (Dickson and Benneh, 2005).

HFL and HFLM production

HFL was produced using natural oviposition method (Charlton *et al*., 2015) using pig manure as substrate. Treatment diets were formulated (Table 1) for grower birds using methods by (Abou-Elezz *et al*., 2012; Safwat *et al*., 2015; Wallace *et al*., 2017). Other chemical analyses were done using methods by A.O.A.C, (1990).

Blood sampling

Blood sampling was done at day 84 (08:00 GMT) prior to feeding. Five growers were randomly picked from each dietary treatment and 5 ml blood was aseptically drawn from the left jugular vein using disposable 5 ml plastic syringe and dispensed into labelled vacuum tubes containing EDTA as anticoagulant, 2 ml of blood was transferred to be used for enumeration of whole blood counts. The remaining 3 ml of blood was transferred into vacuum container tubes laced with gel for blood chemistry analyses.

Blood characterization assays

Erythrocytes and leucocytes analyses were done using the method by (Schalm *et al.,* 1981). Blood was diluted (1:200) using Natt-Herrick solution and red blood cells (RBCs) were counted using the improved Neubaeur haemocytometer (Schalm *et al.,* 1981). Spectrophotometer, (Cecil 100 series, England) was used to determine haemoglobin (Hb) content at 540nm using Drabkin's solution (Schalm *et al.,* 1981).

Table 1: Composition of experimental grower diets for Coastal Savanna ecotype chickens

Dietary treatments	T1	T2	T3	T4	T5
Maize	65	65	65	64	66
Soybean meal	14	11	11	11	12
Fishmeal	3	2.25	1.5	0.75	0
HFLM	0	0.75	1.5	2.25	3
Lysine	0.15	0.15	0.15	0.15	0.15
Methionine	0.10	0.10	0.10	0.10	0.10
Wheat Bran	17.0	17.0	17.0	17.0	17.0
Common salt	0.25	0.30	0.30	0.30	0.30
Oyster shell	2.2	2.15	2.15	2.15	2.15
Dicalcium phosphate	1	1	1	1	1
Premix	0.3	0.3	0.3	0.3	0.3
Nutrient composition analysed					
ME	11.43	11.43	11.48	11.51	11.50
CP	15.62	15.52	15.42	15.32	15.60
C.fa	3.61	3.51	3.42	3.33	3.21
Crude fibre	3.69	3.71	3.74	3.76	3.80
Lysine	0.77	0.77	0.79	0.78	0.81
Methionine	0.34	0.34	0.35	0.35	0.36
Calcium	1.19	1.16	1.15	1.14	1.13
P. Available	0.38	0.37	0.36	0.36	0.35
Sodium	0.16	0.17	0.17	0.17	0.17

Duplicate capillary tube method was used to determine packed cell volume (PCV). Tubes containing blood samples were centrifuged (Model MB) and read with a Hawksley haematocrit reader (Cheesbrough, 1998). Leucocytes were determined by preparing thin blood smears, stained with Giemsa, and the preparation observed microscopically under oil immersion. A minimum of 200 leucocytes were counted to determine differential leucocyte values.

Blood chemistry assay

Blood samples were centrifuged at 3000 rpm for 5 min and supernatant collected for preparation of assays. The assays for lipids, proteins and enzymes were produced using reagent kits (Labcare Diagnostics, India) and the target indices quantified using automatic spectrophotometer (Mindray Bio-Medical Electronics, China).

Statistical analysis

Data generated were subjected to analysis of variance (ANOVA) using GenStat (Version 12.0). Significant means were separated using the least significant difference (LSD) method.

RESULTS AND DISCUSSION

Table 2 shows the haematogram and leukogram summaries of effects of HFLM replacement of fishmeal on grower phase of the indigenous chicken (Coastal Savanna ecotype). The hematological profile is an important indicator of the health status of animals and should therefore be influential in the diagnosis and prognosis of diseases (Elim *et al*., 2014b). There were no significant differences in the RBC and its differentials ($p > 0.05$) suggesting that HFLM at all levels of inclusion were good in erythropoiesis comparatively to fishmeal as far as the grower phase of the Coastal Savanna Ecotype chicken were concerned. Hence any level of replacement of fishmeal with HFLM would not adversely affect RBC formation in the growth phase of the Coastal Savanna ecotype chicken.

No variation occurred in the WBC and its differential in all treatment groups ($p > 0.05$). Values observed during this study (4.316-4.598 X 10^9/L), were higher than the peak value of 31.3 x 10^9/L observed for domestic chicken in Nigeria, (Durotype *et al*., 2000). Blood count values of animals describe the totality of the animal's age, mutation, degree of physical activity, sex, environmental factors and health status (Elim *et al*., 2014b).

Table 3 presents the influence of HFLM on serum concentration of lipids, electrolytes, some

Table 2: Effect of HFLM on hematology of Coastal Savanna ecotype chicken

Parameter	% HFLM replacements							
	0	25	50	75	100	SED	LSD	P value
RBC(x10^{12} /L)	1.784	1.974	2.092	1.804	1.926	0.1968	0.4173	0.526
Hb (g/dL)	9.484	9.482	9.536	9.436	9.484	0.1574	0.3338	0.981
PCV	27.80	29.60	29.80	29.80	29.40	1.701	3.606	0.742
WBC (X10^9/L)	4.316	4.598	4.318	4.586	4.336	0.2194	0.4650	0.485
Monocytes %	0	0.20	0.60	0.20	0	0.400	0.848	0.572
Eosinophils %	1.40	0.80	0.40	0.60	0.20	0.987	2.092	0.696
Basophils %	2.00	0.90	1.00	1.60	0.40	0.951	2.016	0.376
Lymphocytes %	61.4	55.8	57.6	55.4	56.0	4.79	10.15	0.713
MCHC	34.37	33.31	34.33	32.72	35.14	1.981	4.900	0.761
MCH	53.9	52	49.3	55.9	54.4	5.22	11.07	0.750
MCV	551	174	133	138	164	243.9	517.1	0.400

RBC red blood cell, *Hb* haemoglobin, *PCV* packed cell volume, *WBC* white blood cell, *MCHC* mean cell haemoglobin concentration, *MCH* mean cell haemoglobin, *MCV* mean cell volume

Table 3: Effect of HFLM on serum concentration of lipids electrolytes metabolites and enzymes

Parameter	% HFLM replacements							
	0	25	50	75	100	SED	LSD	P
Total Chol (mmol/L)	114.3	141.9	137.6	130.2	128	14.83	31.43	0.429
Triglycerides (mmol/L)	63.1	53.5	53.8	65.5	65.4	8.04	17.04	0.351
Creatinine (mmol/L)	0.460^{a}	0.320^{b}	0.320^{b}	0.32^{b}	0.40^{ab}	0.048	0.101	0.028
Urea (mmol/L)	15.9^{ab}	29.1^{a}	22.3^{a}	22.5^{a}	0.4^{b}	7.81	16.57	0.021
Uric Acid (mmol/L)	1.72	1.12	1.28	1.50	1.40	0.318	0.675	0.431
Na+	150.9	147.2	150.8	147.3	146.3	3.43	7.28	0.551
K+	2.78	3.2	3.26	3.10	2.74	0.397	0.842	0.581
Cl^-	108	115	291	115	115	114.1	241.8	0.451
ALT (µ/L)	3.20	5.80	5.20	5.80	3.10	2.286	4.845	0.595
AST (µ/L)	134.6	124.4	123.8	126.2	137.6	18.95	40.18	0.921
ALP (µ/L)	602^{a}	256^{d}	401^{cd}	521^{ab}	420^{bc}	72	152	0.002
D.BIL (µmol/L)	0.18	0.16	0.16	0.16	0.16	0.03098	0.06568	0.952
T.BIL (µmol/L)	0.300	0.300	0.280	0.300	0.400	0.081	0.172	0.606
ALB (g/L)	28.28	28.84	25.18	26.38	25.34	2.506	5.312	0.488
TP (g/L)	56.3^{a}	48.7^{ab}	39.1^{bc}	28^{c}	35.6^{bc}	8.40	17.80	0.031

^{abc} *Means in a row with the same or no superscript are not significantly different (p>0.05) Tot Chol total cholesterol, ALT alanine transaminase, AST aspartate transaminase, ALP alanine phosphate, T. Bil total bilirubin, D. Bil direct bilirubin, ALB albumin, TP total protein.*

metabolites and enzymes. Total cholesterol, tryglycerides, uric acid, ALT, AST, did not vary significantly for all the five treatments ($p > 0.05$). However, ALP differed among the treatments ($p < 0.05$) so was total protein (TP) ($p < 0.05$).

The values of ALT, AST, ALP obtained (Table 3) agreed with those obtained for broilers as reported by Abdi-Hachesoo *et al*., (2011) but lower than values obtained for indigenous chickens in Saudi Arabia (Ibrahim *et al*., 2012). ALT, AST and ALP biomarkers describe the well-being of some vital organs in animals (Gowda *et al*., 2010). ALT and AST are the most commonly used indicators of liver damage. These enzymes are normally found in damaged liver cells but leak out of these cells into the blood. ALP levels in the blood may be used to describe the state of the liver, whether there is an obstruction in the biliary system. Its elevation in the blood is an indication of biliary cirrhosis or biliary tumors. BIL (T. BIL and D. BIL) levels did not differ significantly ($p>0.05$). BIL is produced from breakdown of heme in RBC. A healthy liver processes BIL for removal from the blood leaving a small amount in the blood of healthy individuals. Increase level of BIL in the blood therefore is a sign of defective liver. ALB is a major protein formed by the liver. When the liver is damaged the level of ALB in circulation in the blood decreases. ALB levels for the dietary treatments were not significantly different ($p>0.05$). Kidney wellbeing is measured by its ability to remove from circulation poisonous metabolic products such as urea and creatine. Urea is produced in the liver as a result of protein break down and is eliminated by the kidney. Urea levels for the dietary treatments did differ significantly ($p<0.05$), (Table 3). Dietary treatment T5 was associated with the least amount (Table 3). Dietary treatments T1 and T2 with high fishmeal inclusions emerged with high levels of urea probably due to the high TP values.

Creatine is derived from breakdown of muscle protein that is eliminated by the kidney. High level of creatine in blood is an indication of kidney impairment. Creatine levels differed significantly for the dietary treatments ($p<0.05$), Table 3.

Serum electrolytes, Na^+, K^+ and Cl^- did not differ

significantly ($p > 0.05$). Except Cl^-, Na^+ and K^+ values agreed with results by Ibrahim *et al*., (2012) for indigenous chicken in Nigeria.

Total serum protein concentration is an indicator of the adequacy of protein quality and quantity in the diet (Ibrahim *et al*., 2012). Fishmeal alone or in combination with 25%, 50% HFLM or HFLM alone (100%) should be enough to provide adequate protein for the growing Coastal Savannah Ecotype chicken (Table 3), maintaining in addition liver, kidney and bird physiological ionic balance.

CONCLUSION

Results of this study showed that HFLM can safely partially or wholly replace fishmeal as protein source for the local grower birds as it did not cause any physiopathological irregularities in the grower phase of the Fulani ecotype chicken. These levels had no adverse effects on haematopoiesis, serum electrolytes, kidney, liver and lymphoid integrity and were able to satisfy the protein needs of the birds.

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