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Haematological parameters, lipid and hormonal profile of heat stressed broilers fed beniseed (*sesamum indicum*) oil

Oganija K. Y.[1] and Apata D. F. [1]

[1]*Department of Animal Production, Fisheries and Aquaculture, Kwara State University, Malete, Nigeria*

***Corresponding Author**: oganijakaothar@gmail.com* ***Phone number**: +2348065470007*

***Target Audience:** Researchers, Poultry farmers, Animal Scientists, Graduate students*

Abstract

High ambient temperature (above 30°C) in the tropics has been reported to cause negative effect on growth, feed efficiency and survivability of chicks in poultry production leading to increased cost of production with consequent low profit for farmers as well as impacting negatively on the welfare of the animals. The effect of Sesamum indicum oil on haematological parameters and lipid profile of heat stressed broilers was examined in this study. One hundred and sixty day old Ross broiler chicks were assigned in a completely randomized design to five treatments and four replicate groups of eight (8) chicks each which were fed ad libitum for 42 days. The diets were formulated to meet the nutrient requirement of the birds. Sesamum indicum (SSO) replaced dietary maize at 0, 2, 4, 6 or 8% on equi-energy basis throughout the feeding trial. From day 28 to 42, all broilers were challenged by heat stress of 29±1°C for 10h per day. Most of the hematology parameters measured were unaffected by SSO diets, except for heterophils, lymphocytes and their ratio at 4% SSO diet which thus showed superiority in coping with heat stress.

***Keywords:** Heat stress; broiler; Sesamum indicum oil; hematology; lipid profile*

Description of Problem

Heat stress occurs in poultry when they have negative balance between the body heat production and loss to the surrounding environment. This imbalance results from variations of a combination of environmental factors such as sunlight, thermal irradiation, air temperature, humidity, movement and characteristics of the animal which include species, metabolism rate, and thermoregulatory mechanisms. This has been a major factor limiting optimum poultry productivity in hot climatic environment (1). However, the effects of environmental seasonality and the inadequacy of facilities to ensure appropriate temperature ranges expose the broilers to heat, thus, impairing their genetic productivity as such, there are significant economic losses to the poultry sector (2) and also the welfare of the animals. The blood system is also particularly sensitive to changes in temperature and an important indicator of physiological responses of broilers to stressors. Quantitative and morphological changes in blood cells are associated with heat stress, reflected in variations in hematocrit values, number of circulating white blood cells, content of erythrocytes and hemoglobin in the red blood cell (3). It is believed that these series of events will release corticosterone and reduced lymphocytes thereby contributing to reduce productivity. According to (4) under stressful situations with release of adrenocorticotropic hormone (ACTH), there is a reduction in the number of circulating lymphocytes, contributing to the increase of the heterophil/lymphocyte ratio. In addition to that, the release of corticosterone may cause involution of lymphoid tissue (thymus, spleen and cloacal bursa) and the suppression of humoral immunity and the cell-mediated immunity (5). Consequently, the broiler

becomes more susceptible to acquiring secondary illnesses.

Heat stress generally causes several physiological changes to maintain body temperature causing reduced immune response (6). During exposure to heat stress, broilers use several mechanisms to dissipate excess heat, and the high temperature is the most important physical factor (7). Reduced feed intake is the first factor used by the animal, aiming to reduce thermogenesis of food metabolism. Simultaneously, water intake increases followed by gasping, production of glucocorticoids and catecholamines, and the metabolism reduction with decrease in thyroid hormones which are important growth promoter in broilers (triiodothyronine (T3) and thyroxine (T4),) while other hormones such as corticosterone, increases (8, 9).

Table 1: Composition of starter diet (%) (0-21 day)

	Level of Sesame Seed Oil (SSO) %				
Ingredients	0	2	4	6	8
Maize	51.3	46.1	41.0	35.9	30.7
Sesame seed oil	-	2.00	4.00	6.00	8.00
Wheat offal	7.0	7.0	7.0	7.9	9.0
Soya bean meal	19.0	20.0	21.0	21.3	22.0
Groundnut cake	16.8	16.8	16.8	17.0	17.4
Fishmeal	2.00	2.0	2.00	2.00	2.0
Oyster shell	1.00	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20
*Vitamin/min premix	0.25	0.25	0.25	0.25	0.25
Table Salt	0.20	0.20	0.20	0.20	0.20
Fine Sand	-	22	43	60	70
Calculated analysis					
ME Kcal/kg	2926.01	2951.24	2979.91	3011.79	3060.05
Crude protein %	23.12	23.00	23.01	23.13	22.90
ME/CP	126.58	127.80	129.12	130.95	131.83
Crude fibre	3.72	3.68	3.64	3.64	3.70
Ether extract	4.06	5.99	7.71	9.67	11.45
Lysine	1.37	1.38	1.40	1.4	1.43
Methionine	0.59	0.58	0.59	0.58	0.60
Calcium	1.29	1.30	1.30	1.3	1.44
Available phosphorus	0.57	0.58	0.58	0.58	0.58

* Vit. A,, 12,500 IU; Vit. D3, 2,500 IU; Tocopherols, 50.00mg; Vit. K3, 2.50mg; Folacin, 0.25mg; Vit B1 3.00mg; Vit B2 5,000 mg; Niacin, 40mg; Calcium Panthothenate, 10mg; Vit B6, 6.00mg; Vit B12, 0.25mg; Biotin, 0.08mg; Manganese, 100mg; Zinc, 45mg; Folic acid, 1.00mg; Iron, 50mg; Choline chloride, 300mg; Copper, 2.00mg; Iodine, 1.55 mg; Cobalt, 0.25 mg; Selenium, 0.01; Antioxidant, 200mg.

Materials and Methods

Birds and dietary treatments

A total of 160-day old Ross 308 broiler chicks were used in a 42-day feeding trial in a completely randomized design to assess the haematology, serum biochemistry and hormonal profile of broilers fed varying dietary inclusion levels of sesame oil. The levels were 0%, 2, 4, 6 and 8% each of starter and finisher diets (Tables 1 and 2). The dietary treatments

were further replicated 4 times with 8 birds per treatment. Feeding and water supply to the birds were ad libitum while other routine management practices were observed. On 28 day of the experiment, heat stress was induced by maintaining the temperature of 29°C±1°C for 10hrs daily (8a.m. to 6p.m.) till the 42day of the experiment when responses were monitored accordingly.

Table 2: Composition of finisher diet (%) (21-42day)

	Level of Sesame Seed Oil (SSO) %				
Ingredients	0	2	4	6	8
Maize	50.0	44.8	39.7	34.5	29.4
Sesame seed oil	-	2.00	4.00	6.00	8.00
Wheat offal	18.5	18.5	18.5	19.0	19.7
Soya bean meal	15.6	16.6	17.6	17.6	18.0
Groundnut cake	11.0	11.0	11.0	12.0	13.0
Fishmeal	1.0	1.0	1.0	1.0	1.0
Oyster shell	1.00	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20
*Vitamin/min premix	0.25	0.25	0.25	0.25	0.25
Table Salt	0.20	0.20	0.20	0.20	0.20
Fine Sand	-	22	43	60	70
Calculated analysis					
ME Kcal/kg	2822.90	2848.13	2876.8	2910.78	2962.74
Crude protein %	20.13	20.11	20.09	20.16	20.46
ME/CP	140.24	141.65	143.18	144.35	144.84
Ether extract	3.9	5.79	7.52	9.58	11.44
Crude fibre	4.12	4.11	4.07	4.06	4.09
Lysine	1.19	1.20	1.21	1.22	1.22
Methionine	0.55	0.54	0.54	0.54	0.56
Calcium	1.23	1.23	1.23	1.23	1.38
Available phosphorus	0.55	0.55	0.55	0.55	0.62

* Vit. A,, 12,500 IU; Vit. D3, 2,500 IU; Tocopherols, 50.00mg; Vit. K3, 2.50mg; Folacin, 0.25mg; Vit B1 3.00mg; Vit B2 5,000 mg; Niacin, 40mg; Calcium Panthothenate, 10mg; Vit B6, 6.00mg; Vit B12, 0.25mg; Biotin, 0.08mg; Manganese, 100mg; Zinc, 45mg; Folic acid, 1.00mg; Iron, 50mg; Choline chloride, 300mg; Copper, 2.00mg; Iodine, 1.55 mg; Cobalt, 0.25 mg; Selenium, 0.01; Antioxidant, 200mg.

On the 42day of the experiment, five birds per treatment were randomly selected for blood collection. Blood was collected through neck bleeding. Blood of each bird was collected into two bottles each EDTA coated for hematology and uncoated for lipid profile analysis. Blood in the uncoated bottle and that for hormonal assay was centrifuged at 3000 rpm for 15 min. Plasma and serum were stored at -20ºC until ready for analysis to determine the total cholesterol TC, low density lipoprotein (LDL), high density lipoprotein (HDL) and Triglyceride (TG) using the instruments and kits of Hitachi-911 (Boehringer Mannheim, Germany).

Table 3 Effect of Dietary Sesame Seed Oil on Hematological Indices of Heat Stressed Broilers

Haematological Parameters	Level of SSO in the diets, %					
	0	2	4	6	8	SEM±
PCV (%)	31.25	30.50	29.00	30.00	26.75	0.93
RBC (×10^{12}/L)	5.05	4.80	4.32	4.64	4.40	4.64
WBC(×10^{9}/L)	6.38	5.83	6.43	5.18	5.55	0.29
HGB (g/dl)	9.25	8.80	8.33	8.63	7.85	0.31
MCV (Fl)	62.00	63.50	67.50	65.00	60.50	1.10
MCH (Pg)	18.35	18.45	19.33	18.70	17.73	0.27
MCHC (g/dl)	29.55	28.80	28.48	28.78	29.38	0.28
PLT (×109/L)	288.50	292.75	263.25	282.00	234.25	13.36
Heterophils (%)	35.50^{b}	42.00^{b}	25.25^{a}	34.50^{b}	39.25^{b}	1.63
Lymphocytes (%)	61.25^{abc}	54.50^{a}	68.25^{c}	63.00^{bc}	58.75^{ab}	1.41
Monocytes (%)	2.75^{ab}	2.50^{ab}	4.00^{b}	2.25^{ab}	2.00^{a}	2.70
Eosinphils (%)	0.50^{a}	1.00^{a}	2.50^{b}	0.25^{a}	0.00^{a}	0.24
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00
Heterophil: Lymphocte	0.5913^{bc}	0.7813^{c}	0.3728^{a}	0.6696^{ab}	0.5944^{bc}	0.040

^{abc} means on the same rows having different superscripts were significantly different ($p<0.05$) SEM = Standard error of mean

PCV=Packed cell volume, RBC=Red blood cell, WBC=White blood cell, HGB= Haemoglobin, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular hemoglobin concentration, PLT= Platelet

Table 4 Effect of Dietary Sesame Seed Oil on Lipid Profile of Heat Stressed Broilers

	Level of SSO in the diets, %					
	0	2	4	6	8	SEM±
Total cholesterol (mmol/l)	5.225	4.750	4.300	5.650	5.800	.2488
Triglyceride (mmol/l)	0.725^{b}	0.700^{b}	0.850^{b}	0.400^{a}	1.100^{c}	.0605
LDL (mmol/l)	3.275	3.900	3.250	4.425	4.425	.2485
HDL (mmol/l)	1.050^{b}	0.500^{a}	0.650^{ab}	0.450^{a}	0.675^{ab}	.0726

^{abc} means on the same rows having different superscripts were significantly different ($p<0.05$)

SEM = Standard error of mean

LDL = Low density lipoprotein, HDL= High density lipoprotein

Table 5 Effect of Dietary Sesame Seed Oil on Hormonal Profile of Heat Stressed Broilers

Hormone	Level of SSO in the diets, %					
	0	2	4	6	8	SEM±
TRIIODOTRIONINE T3 (nmol/l)	8.94	4.5	7.09	5.72	4.35	0.975
TYROXINE T4 (nmol/l)	13.35	21.17	14.40	13.18	13.85	2.36

SEM = Standard error of mean

The direct measurements of erythrocytes values; {Haemoglobin (Hb), Packed cell volume (PCV), Red blood cells (RBC)}, Mean Corpuscular Haemoglobin (MCH) was done. Platelet and differential counts (neutrophils and lymphocytes) were analyzed as described by (10) For the relative and absolute automated blood cell count (A-diff), 500µL blood was

analyzed with the automated haematology analyzer SYSMEX KX21-JAPAN at the Veterinary laboratory of University of Ilorin.

Hormonal assay (thyroxine and triiodothyronine) was done using the kit of ichroma™. Frozen plasma was cooled at room temperature. 75µl of sample was transferred using a micropipette to tube containing solution A packed by the manufacturer. This was mixed well by pipetting 10 times. 75µl of solution B was taken and added to the mixture of solution A and sample using a new tip. The lip was closed and thoroughly shaken for about 10 times. The mixture was incubated at room temperature for 8minutes. 75µl of the mixture was pipette and loaded into the sample well on the cartridge. The sample-loaded test cartridge was inserted into the slot of the i-Chamber and left for 8 minutes. Readings were then taken.

Response data were subjected to one-way analysis of variance using Generalized Linear Model (GLM). Significant mean differences were separated by Duncan's multiple range test.

Results

There was no significant difference ($p>0.05$) observed on all the parameters measured except for differential counts (heterophils, lymphocytes, monocytes and eosinophils). For heterophils, 4%SSO had the least value while all other dietary treatments did not differ significantly ($p>0.05$) among each other. 4%SSO gave a higher value for lymphocytes, monocytes and eosinophils while all other dietary treatments did not differ significantly ($p>0.05$) among each other. Significant difference ($p<0.05$) was also observed between the group in heterophil:lymphocyte ratio in which 2% SSO had the highest value and 4% SSO the lowest (Table 3). There was no significant effect of the dietary treatment on the total cholesterol and LDL (Table 4). On the other hand, both triglyceride and HDL were significantly ($p<0.05$) different among the treatments. Highest triglyceride value was recorded in 8%SSO, followed by 0, 2 and 4%SSO while 6%SSO had the least value. 0%SSO had the highest HDL value, followed by 2 and 6%SSO, 4 and 8%SSO did not differ significantly ($p>0.05$) from 0, 2 and 6%SSO (Table 4).

No significant ($p>0.05$) difference was observed in both hormones (triiodotrionine (T3) and thyroxin (T4) (Table 5).

Discussion

(11) reported that diets have significant influence on haematological variables. The haematological parameters for the treatments were similar for RBC, PCV, WBC, HGB, MCV, MCH, MCHC. All others heterophils, lymphocytes, monocytes and eosinophils were significantly different. The values of PVC, falls within the normal range (26.56%-38.0%) for chickens as reported by (12, 13, 14) this indicates the good health status of the birds. The results of Hb follows the same pattern as PCV and also falls within normal range (6-13%) (15), this shows that SSO based diets are nutritionally adequate to meet the protein requirement of the birds. This result also suggests that the blood of the birds had an appreciable oxygen carrying capacity which shows that nutrient transport by the blood was not impaired by feeding SSO.

The response measurement of stress-induced immune alteration to heat exposure by the use of Heterophils and Lymphocytes as well as their ratio (H/L) is well documented (16, 17, 18). This current research also employs the same (16) as a measure of stress response, the result showed that only 4% SSO had the best ratio of Heterophils and Lymphocytes (0.3728)

SSO is a good source of Poly Unsaturated Fatty Acid (PUFA) and thus have cardio-protective effect (19) by increasing HDL and reducing LDL. This present study did agree with the result (20) who demonstrated that the regular consumption of w-3 PUFA is efficient in reducing total cholesterol, cholesterol fractions and triglycerides.

Dietary SSO did not cause any significant

effect on the hormones measured (T3 and T4) although in-consistency was observed on the hormones. It was earlier hypothesized that since both hormones are growth hormones and heat stress had been proven to have a negative effect on growth thus changes should be observed on the hormone however, no change was observed. This might be due to the effect of the oil for being able to counter the negative effect of heat stress although the mechanism of that is beyond the scope of this current research.

Conclusion and Applications

1. Sesame seed oil as a substitute for maize on heat stressed broilers did not have deleterious effects on the birds.
2. Using H/L as measurement of heat stress, the 4% SSO is the best inclusion levels for alleviation of heat stress in broiler chickens.

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