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Haematoprotective Effect of Fried Dung Beetle Larval Supplemented Diet in Rats

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Abstract

Background and Aim: Blood act as a pathological reflector of the status of exposed animals to toxicants and other conditions. The research investigates the haematological parameters of rats fed with fried dung beetle larval supplementation.

Materials and Methods: The experimental diets were formulated such that soy beans were replaced with Fried Dung Beetle Larvae (FDBL) at 25%, 50%, 75% and 100% dietary levels. The control group was fed with 100% soy bean (0% FDBL). After 28 days, the values of RBC, WBC, MCH, MCHC, MCV, Platelets, lymphocytes, neutrophils, eosinophils counts were estimated using the automated haematologic analyzer.

Results: Consumption of fried dung beetle larval supplemented diet by albino rats did not affect the values of RBC, WBC, MCH, MCHC, MCV, platelets, lymphocytes, neutrophils and eosinophils counts of rats to any significant ($P>0.05$) degree when compared with the control. However, there was an increased level of basophil at 50%, 75% and 100% diet supplement while there was significant ($P<0.05$) decrease in PCV, haemoglobin and monocytes counts with increased fried dung beetle larval supplementation.

Conclusion: The finding of this study showed that fried dung beetle larval supplementation is relatively non-toxic to haematological indices in rats and can replace soy bean meal in animal diet.

Keywords: Albino Rats; Dung Beetle Larval Supplementation; Heamatological Components.

Introduction

There is much interest in possible replacement of the expensive feed ingredients such as fish meal with insect proteins as an alternative source of animal-derived protein and caloric energy for the use in feed and food value chain [1-4]. Furthermore, insect's high fecundity, multivoltine lifecycle, high feed conversion ratio, omnivorous diet, low substrate, water and space requirements highlight the potential of edible insects to contribute to global food security either via feed or indirectly as food [2.5].

Livestock production consume 85% of global soya production to make concentrated animal feeds and the land used for soya production exhibits well known effects of mono cultured crops including destroyed biodiversity, decreased soil fertility and depleted water resources [6]. Again, prices of soybean and soy oil used for animal diets have increased due to rapid expansion in demand caused by a growing world population. With world prices of feed ingredients increasing, the industry is looking for alternative protein sources.

Hematology is the study of numbers and morphology of the cellular elements of the blood (red cells, white cells, and the platelets) [7,8]. Blood serves as a pathological mirror indicating the conditions of animals exposed to different dietary treatments [9]. Laboratory examination of the blood and other body fluids are vital tools for detecting even the slightest changes due to infections in human and animal body [10,11].

Through haematological analysis, significant physiological, nutritional and pathological information can be obtained with respect to the status of an organism [9]. Haematological constituents are actually correlated with the health status of an animal and are used to examine stress under different conditions [12,13].

Dietary constituents have considerable influence on haematologicalcomponents, thus blood components are commonly used in nutritional assessments and investigation of farm animals [9]. Haematological indices are very essential in monitoring the nutritional quality of feed particularly with feed ingredients that affect the blood as well as the health status of the animals [14]. The aim of this research was to evaluate the effect of fried dung beetle larval supplemented diet on blood constituents of rats.

Materials and Methods

Sample Collection and Identification

Fresh samples of dung beetle larva (*Aphodius rufipes*) average weight 450.9 grams were obtained from Kure market, Minna, Niger State, Nigeria in December 2016 to July 2017. The insects' larvae were handpicked and transferred to sterile perforated containers. The insects were identified and authenticated by an Entomologist in Department of Biological Sciences, Federal University of Technology, Minna.

Sample Preparation

The abdomen of fresh and healthy samples of dung beetle larva were pierced to remove the faeces and washed in sterile hot water before processing by frying in the laboratory at temperature of 60˚C for 10 minutes [15]. The fried sample was then pulverized using a sterile mortar and pestle and labeled FDBL.

Experimental Animals

Twenty-fivehealthy albino rats of weight (70 grams) were purchased from Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria. The animals were housed and cared for in accordance with Good Laboratory Practice (GLP) regulations of WHO [16]. The principle of laboratory animal care (National Institute of Environment and Health Services, NIEHS, 1985) was also followed throughout the study.

Experimental Protocol

Feeding experiment was conducted according to the method described by [17]. Five experimental diets were formulated such that the quantity of soy bean was reduced while Fried Dung Beetle Larval Meal (FDBL) was increased until soy bean was finally replaced with FDBL at 25%, 50%, 75% and 100% dietary levels respectively. The control groups were fed with 100% soy bean meal and 100% FDBL. Other ingredients remained the same in all the diets as shown in (Table 1). The rats were divided into five groups of 5 rats each and randomly assigned to the five diets. Feed and water were provided ad-libitum and other routine experimental animals management practices maintained. Rats were weighed at the beginning of the experiment and thereafter weekly. Quantity of feed intake

was determined by obtaining the difference between the quantity of feed given and the quantity left over the next morning.

Ingredients	Groups				
(g)	1	2	3	4	5
Maize	450	450	450	450	450
Maize offal	350	350	350	350	350
Soy bean	100	75	50	25	00
FDBL	00	25	50	75	100
Palm kernel cake	40	40	40	40	40
Bone meal	20	20	20	20	20
Minerals	7.5	7.5	7.5	7.5	7.5
Methionine	2.5	2.5	2.5	2.5	2.5
Lysine	2.5	2.5	2.5	2.5	2.5
Vitamins	25	25	25	25	25
Salt	2.5	2.5	2.5	2.5	2.5

Table 1: Composition of experimental diets per gram utilized in the feed trial (grams).

FDBL-Fried dung beetle larva meal

Total-1000grams

Group 1: 100% soy bean and 0% FDBL
Group 2: 75% soy bean and 25% FDBL
Group 3: 50% soy bean and 50% FDBL
Group 4: 25% soy bean and 75% FDBL
Group 5: 0% soy bean and 100% FDBL

Estimation of Hematological Parameters

At the end of 4 weeks feed trial, blood samples were collected as described by [18]. Briefly, the animals were anaesthetized with diethyl ether. The blood samples collected into EDTA bottles were analyzed immediately for haematological indices. The hematological components including Haemoglobin (HB), Packed Cell Volume (PCV), Red Blood Cells (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin(MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cells (WBC), Platelet Count (PLT) and differential count (basophils, lymphocytes, eosinophils, monocytes and neutrophils) were determined using the automated haematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by [19].

Statistical Analysis

Results were expressed as mean values ± Standard Deviation (S.D). Within groups, comparisons were performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups were assessed by Duncan's Multiple Range.

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Results

Effect of Dung Beetle Larval Supplemented Diet on Hematological Parameters of Rats

The results of the effects of fried dung beetle larval supplemented (25%, 50%, 75% and 100% protein) diet on hematological parameters in rats are shown in (Table 2). The values of red blood cells, white blood cells, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume and platelet counts were not significantly ($P>0.05$) altered by the replacement of soy proteins with fried dung beetle larval (25%, 50%, 75% and 100 %) supplemented diet when compared with the control. However, packed cell volume and haemoglobin counts were significantly ($p<0.05$) lowered in rats fed 100 % fried dung beetle larval supplemented protein based diet.

Treatment (%)	PCV (%)	HB (gram/deciLiter)	WBC ($x10^9$/Liter)	RBC ($x10^9$/Liter)	PLT ($x10^6$/Liter)	MCV (Fluid/Liter)	MCHC (gram/deciliter)	MCH (picogram)
Control	43.00 ± 3.60^a	14.30 ± 1.17^a	5.13 ± 1.05^a	4.93 ± 0.76^a	278.67 ± 47.59^a	90.66 ± 12.05^a	27.00 ± 7.00^a	332.00 ± 1.00^a
25	40.33 ± 4.16^{ab}	13.43 ± 1.40^{ab}	5.26 ± 1.04^a	4.70 ± 1.21^a	300.33 ± 16.74^a	90.33 ± 26.63^a	30.06 ± 8.90^a	332.67 ± 0.57^a
50	40.66 ± 3.78^{ab}	13.56 ± 1.25^{ab}	5.73 ± 0.55^a	5.00 ± 1.04^a	269.67 ± 33.94^a	69.00 ± 7.54^a	22.96 ± 2.3^a	333.00 ± 1.00^a
75	36.33 ± 3.21^{ab}	12.10 ± 1.05^{ab}	5.36 ± 0.85^a	5.03 ± 1.13^a	304.33 ± 45.23^a	82.33 ± 12.05^a	27.50 ± 3.97^a	333.33 ± 0.57^a
100	34.00 ± 4.00^b	11.33 ± 1.35^b	5.33 ± 0.28^a	4.43 ± 0.51^a	303.33 ± 14.43^a	83.00 ± 13.22^a	27.60 ± 4.40^a	339.67 ± 10.06^a

Values are mean ± SD for n=3. Mean values with the same superscript on the same column are not significantly different from each other ($p<0.05$).WBC=White Blood Cells; RBC=Red Blood Cells; PCV=Packed Cell Volume; HB=Haemoglobin; MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration MCV=Mean Corpuscular Volume; PLT= Platelets Count.

Table 2: Effect of fried dung beetle larval supplemented diet on hematological parameters.

Effect of Dung Beetle Larval Supplemented Diet On Differential Count of Rats

The results of the effects of fried dung beetle larval supplemented (25%, 50%, 75% and 100% protein) diet on differential count in rats are shown in (Table 3). The values of differential white blood cell counts (lymphocytes, neutrophil, eosinophils) were not significantly ($P>0.05$) altered by the replacement of soy proteins with fried dung beetle larval (25%, 50%, 75% and 100 %) supplemented diet when compared with the control. The values of basophils were not significantly ($p>0.05$) altered by the replacement of soy proteins with fried dung beetle larval (25%) supplemented diet when compared with the control while rats that were fed (50%, 75% and 100%) had significant ($p<0.05$) increase in basophils counts. However, monocyte counts significantly decreased with increase fried dung beetle larval supplementation.

Treatments (%)	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Control	46.66±5.85^{a}	44.33±6.02^{a}	4.66±1.52^{a}	3.33±0.57^{a}	0.00±0.00^{b}
25	46.33±8.08^{a}	44.66±9.07^{a}	4.00±1.00^{a}	3.33±1.15^{a}	0.00±0.00^{b}
50	48.00±7.2^{a}	45.66±9.8^{a}	3.66±1.15^{ab}	2.66±1.52^{a}	0.33±0.00^{a}
75	45.66±9.29^{a}	48.66±9.29^{a}	3.33±1.15^{ab}	2.66±0.57^{a}	0.33±0.00^{a}
100	50.33±2.51^{a}	46.66±2.88^{a}	1.66±1.52^{b}	1.33±1.52^{a}	0.33±0.00^{a}
Values are mean ± SD for n=3. Mean values with the same superscript on the same column are not significantly different from each other ($p<0.05$), DB: Dung beetle larva.					

Table 3: Effect of fried dung beetle larval supplemented diet on differential count in rats.

Discussion

It has been reported that ingestion of dietary constituents has considerable effects on blood composition, thus blood analysis could be considered as the fastest means of evaluating the suitability, quality and toxicity of ingested feed in farm animals [20]. Fried dung beetle larval diets in all treatment groups were well tolerated by the rats and no mortalities were recorded. In the present study, supplementation of fried dung beetle larva at concentration of 25%, 50%, 75% and 100% in the diet of albino rats was accompanied with no significant ($p>0.05$) effect on RBC, MCH, MCV and MCHC. [21] also observed absence of alteration in blood variables in rabbits fed insect fat dietary supplementation. The lack of significant biological differences in many of the blood variables across the dietary treatments in the study suggest that dung beetle larval supplemented diet could be safely incorporated into the animal diet without having detrimental effect on their health.

White blood cells defend the body against infections or any foreign body. The insignificant ($p>0.05$) alteration on WBC and factors relating to it (lymphocytes, neutrophils, eosinophils) recorded in this research may indicate adequate immune response of the animals. It appeared that fried dung beetle larval supplementation did not provoke any immunological distress, thus no activation of leucopoiesis [22]. The results obtained in this study is in agreement with that of [23] who reported that total and differential leucocytes count did not differ when rabbits were fed a diet containing up to 30% of Bt cotton seed.

Red Blood Cells (RBCs) are the carrier of haemoglobin in the blood. Haemoglobin reacts with cellular oxygen to form oxyhemoglobin during respiration process [14]. Studies have revealed that RBCs transport oxygen and carbon dioxide throughout the body [13]. The RBC values recorded in this study were not significantly affected by the dietary treatments. This is an indication that the animals in all dietary groups have maintained normal respiratory capability and did not significantly suffer from any haematological disorder such as erythropoiesis. The finding of this study corroborated the report of [24] who fed mice with diet containing 60% rice genetically modified to express Bt and epsps proteins. He reported non-significant alterations in mice erythron values.

Blood platelets are significantly involved in blood clotting in animals. High blood platelet concentration could be linked with iron deficiency (anemia) and chronic feeding. Elevated platelet values can also be measured in the condition of cold stress. Low platelet count is an indication of severe allergic reaction, excessive bleeding, aplastic anemia, systemic bacterial or fungal infections [14]. In this study, platelet counts were not significantly ($p>0.05$)

altered by the replacement of soy proteins with fried dung beetle larval supplemented diet when compared with the control.

Similar to our findings, Walsh and co-workers did not find significant biological difference in the platelet values when modified maize expressing crylab proteins were fed to sows. [25] also reported normal platelet values when glyphosate-resistant transgenic soybean was supplemented in diet of Spraquey Dawley (SD) rats. However, the low Packed Cell Volume and Haemoglobin levels at 100% FDBL are undesirable as this indicates erythropoietin inhibitory effect. Thus, increase susceptibility of animals to anemia. The increased level of basophil counts with increased fried dung beetle supplementation suggests allergic reactions in the blood [26]. Findings in this study showed a reduction in haemoglobin concentration with larval supplementation. The reduction may have occurred due to disturbances in osmoregulatory system or oxidative injury to the cell membrane which cause lysis of blood cells [27].

Packed Cell Volume (PCV) is the percentage of Red Blood Cells (RBC) in circulating blood. Decreased PCV indicates loss of red blood cell from any variety of reasons like cell destruction, blood loss and failure of bone marrow production. Since PCV levels reflect the efficiency and extent of oxygen uptake and transfer to tissues, the observed reduction in PCV values in rats suggests different levels of disturbance in osmoregulatory system of the blood cells [27]. The discrepancy in reduced PCV value did not translate to similar variation in RBC obtained in this study. This means that although the effect of feed supplement was statistically significant, the red blood cells were not significantly affected to the extent of bringing about low RBC count (anemia) [27].

Conclusion

Our study highlighted the safety of dung beetle larval supplemented diet on haematological indices in rats.

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Conflict of Interest: The authors declare no conflict of interest.

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