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Evaluation of Nutritional Status of Soldier Ant (*Dorylus spp*) Meal in Partial Replacement for Fishmeal on Some Haematological, Biochemical and Enzymological Parameters of Wistar Albino rats (*Rattus norvegicus*)

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ABSTRACT

An experiment was conducted to evaluate the nutritional and toxic effects of soldier ant meal as a supplement for fishmeal on some haematological, biochemical and enzymological parameters of wistar albino rats. Thirty rats aged 3 weeks, were randomly assigned to the dietary treatments (T) in a complete randomized design with six rats per treatment. The rats were fed with diets containing 0% (T1) (Control), 25 % (T2), 50 % (T3), 75 % (T4) and 100% (T5) soldier ant meal. The experimental diets and drinking water were supplied *ad libitum* throughout the experimental period of three weeks. There were significant reduction ($p<0.05$) among T2, T3, T4 and T5 for red blood cell count (RBC), packed cell volume (PCV) and haemoglobin (Hb ,) mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) compared to the control treatment T1. Also, significant increase in the white blood cell count (WBC) of the treated rats compared to the control treatment was observed. There were significant differences ($p<0.05$) in the biochemical parameters among the treatments and enzymological study showed significant ($p<0.05$) increase in the activities of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in the liver and muscle of T2, T3, T4 and T5 compared to the control treatment. It can be concluded that soldier ant meal has adverse effects on the haematological, biochemical and enzymological parameters of rats.

Keywords: Nutritional Status, Toxic effects, Soldier ant, Fishmeal, Haematological, Biochemical and Enzymological parameters

1. Introduction

Nutrition is the provision to cells and organism, of the materials necessary (in form of food) to support life. The diet of an organism is what it eats, which is largely determined by the perceived palatability of food Shlio and Das reported that most developing countries are confronted with the challenges of acute shortage of animal protein that adversely affects the level of protein intake in the country's population and this consequently leads to unbalance diet and malnutrition [1, 2]. Balance diet is quiet expensive in developing countries such as Nigeria and the abundance and low cost carbohydrate in the country has made it the only assessable and affordable food for the masses especially, the poor who cannot afford the expensive status of protein. According to literature, protein is a vital and important nutrient used in maintaining and repairing tissues for organisms to enable proper growth and development. However, due to the high cost of animal protein, it is therefore imperative to look for alternative source of protein that is cheap and affordable for the masses.

Insects have played an important role in human nutrition as a source of protein for thousands of years [3] has reported that insects are high in proteins ranging between 60%-7o%, energy, and various vitamins and minerals and [4] opined that insects can form 5-10% of annual animal protein consumed by certain indigenous people [5] reported that most species of Orthoptera order, grasshoppers form part of the diet of some ethnic groups in all continents. Most edible insects are cheap, available and can provide a good source of protein and minerals needed to complement cereal-based foods consumed in the developing countries [6, 7] reveals that the level of proteins in the insect species is generally high, above those of traditional sources of protein such as meat, dairy products and some seeds.

As a result, insects are a good alternative as they are consumed all over the world [5].

The proximate analysis of soldier ant according to [8], illustrated that, soldier ant has a protein composition of 12.09%, fat 18.92%; fiber 20.13%, ash 8.54%, moisture 12.62% and carbohydrate 27.72% which invariable makes it a valuable constituent in the formulation of feed to livestock. He also reported that soldier ants showed potentials as good sources of protein, fat, carbohydrates and macro element. However, the nutritional status of soldier ant meal on animals has not been studied in details in Nigeria.

2. Materials and Methods

2.1 Experimental Animal

Thirty apparently healthy Wistar albino rats (*Rattus norvegicus*) of three weeks old purchased from the animal house, University of Ibadan were used for this study. Rats were acclimatized under the laboratory conditions of 25±5 °C and 65±5 °C R.H for one week and given normal commercial feed ration before being subjected to the experimental diets. The rats were randomly assigned to five dietary treatments group with six rats per treatment and each rat was housed in wooden cage compartment measuring 42×29×39 cm. the experimental diets and clean drinking water were supplied to the rats *ad libitum* throughout the experimental period of two weeks. The feed used in this study was formulated (Table 1) and the rats were fed two times a day. Animal experiments and housing procedures were performed in accordance with the principle of laboratory animal care rules and they were approved by the authorities of the University.

Fig 1: Soldier ant trail before the collection

Fig 2: Dried Soldier ant

2.2 Collection of Soldier Ants

Soldier ant collection was based on indigenous knowledge whereby pieces of foam were soaked with palm oil as bait and placed in their path. These greatly attracted the soldier ant and formed a large population around the baited foam and were immediately dropped in a bowl of hot water at 70 °C for 10 minutes and then sieved. The dead soldier ants were sun dried for one week and the dried soldier ants were milled with moultinex electric blender. This method of collection was carried out at different unfumigated area of Ago-Iwoye, Ogun state, Nigeria. This place is situated in Ijebu-North, Ogun, Nigeria, its geographical coordinates are 6° 57' 0" North, 3° 55' 0" East.

Table 1: Weight composition of the formulated feed

Ingredient	Weight (%)
Fish meal	30.00
Groundnut cake	17.98
Wheat offal	19.61
Bone meal	0.50
Soyabean meal	2.00
Mineral premix	0.25
Dicalcium phosphate	0.10
Corn meal	29.56
Total	100%

2.3 Proximate Composition and Anti-nutritional Content of the Experimental Diets

Diets compositions were analyzed before the experiment for crude protein, crude fiber, moisture content, ash content, carbohydrate, dry matter and fat according to the Association of Analytical Chemist Method [9]. Phytate and Flavonoid contents of the diets were analyzed according to the method of wheeler. The procedure of Day [4] was adopted for the analysis of Oxalate and protease inhibitor of the diets and Saponin and Tannin contents of the experimental diets were carried out according to the method of Makkar [12].

2.4 Blood Sample Collections and Hematological Study

Blood samples were randomly collected from three rats per treatment following the termination of the feeding trials. The rats were anaesthetized in desiccators by means of chloroform. Blood samples were collected from each rat separately by cardiac puncture using a 5ml syringe into Lithium heparin anticoagulant bottles for hematological studies by means of Auto hemo-analyser.

2.5 Biochemical Studies
Plasma protein and plasma glucose of the rats were the major biochemical parameters under study. The method of Lowry [13] was adopted to estimate the plasma protein and plasma glucose was estimated by O-toluidine method [14].

2.6 Enzymological Studies
The activities of the enzymes glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were investigated. Following the isolation of the liver and muscles from the rats, 100 mg of each tissue were weighed and homogenized with 2.5ml of 0.25M sucrose solution in ice cold condition [15]. The homogenates were later centrifuged for 20minutes at 600rpm and the supernatant fluid was taken for the estimation of GOT and GPT activities by 2, 4-DNPH method [16].

2.7 Statistical analysis
The data collected throughout the experimental period were subjected to analysis of variance (ANOVA). Duncan's multiple range tests [17] were used to determine difference in mean.

3. Results
Generally, the response of the treatments to the experimental diet was abnormal. Depressed growth and high mortality rates in ascending order of T2 to T5 was observed among the treatments compared to the control group (T1).

The data showing the proximate composition and anti-nutritional contents of the experimental diets are reported in Table 2 and 3 respectively. There were significant difference (P<0.05) in anti-nutritional contents among the experimental diet with diet 5 having the highest phytate, oxalate, saponin, tannin and flavonoid from that of the control diet.

Table 2: Proximate composition of the control and experimental diet

Parameters	Experimental Diets				
	DI	**D2**	**D3**	**D4**	**D5**
Moisture	12.91	13.33	12.20	12.07	14.52
Dry matter	87.04	86.67	87.82	87.93	85.48
Fat	2.67	1.51	1.62	1.87	1.92
Ash	1.06	1.78	2.06	2.28	2.32
Fibre	2.67	2.76	3.98	6.15	6.68
Protein	28.82	27.17	27.45	28.11	28.87
Carbohydrate	51.82	52.95	54.69	55.96	54.45

Where D1 is the control diet with 0% Soldier ant and D2, D3, D4, D5 has 25%, 50%, 75% and 100% Soldier ant respectively. (D = Diet)

Table 3: Anti-nutritional Contents of the Control and Experimental Diets

Parameters	Experimental Diets				
	D1	D2	D3	D4	D5
Phytate	0.82	2.12	2.32	2.51	2.65
Protease	1.07	1.11	1.52	1.65	1.78
Oxalate	1.84	2.05	2.21	2.45	2.57
Saponin	1.22	6.11	6.32	6.51	6.74
Tannin	0.91	3.54	3.92	4.11	4.23
Flavonoid	0.89	2.65	3.04	3.15	3.42

The values of various hematological parameters are presented in Table 3. There were significant reduction (P<0.05) in PCV, RBC and Hb of T2, T3, T4 and T5 from that of control (T1) with T5 registered the highest reduction. There was also significant decrease (P<0.05) in MCV, MCH and MCHC values among the rats treated with the experimental diets, but significant increase (P<0.05) in WBC value was recorded among T2, T3, T4 and T5 compared to the control (T1).

Table 4: Effects of Soldier ant meal on heamatological indices of control and treated rats

Parameters	Treatments				
	T1	**T2**	**T3**	**T4**	**T5**
PCV	38.21	26.21	24.28	24.50	22.76
RBC	8.35	7.45	7.19	7.32	6.79
WBC	9.13	23.29	17.12	15.21	19.27
Hb	12.81	7.94	7.16	7.27	5.62
MCV	51.40	41.58	40.42	40.24	33.51
MCH	15.33	12.91	10.40	10.36	9.62
MCHC	29.81	22.84	20.50	19.90	19.32

Biochemical results showed a significant increase (P<0.05) in plasma glucose in treated rats when compared to the control group (Fig. 1). On the contrary, plasma protein level decreased significantly in treated rats when also compared to the control group (Fig. 1).

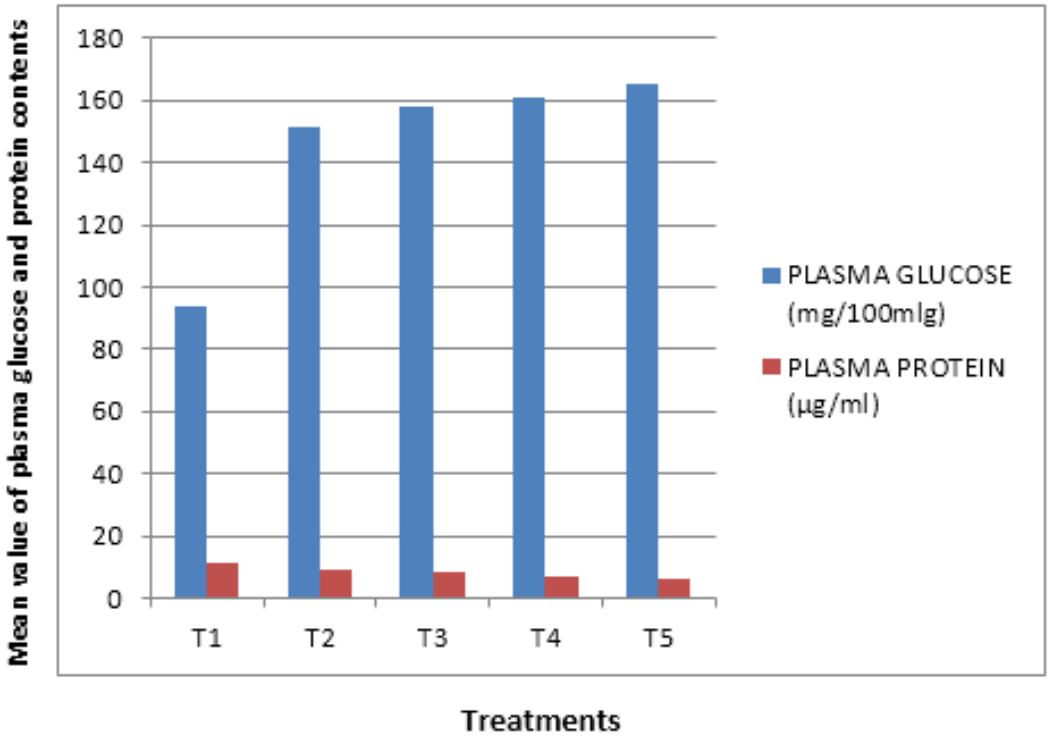

Fig. 1: Changes in plasma glucose and protein content of control and treated rats, where T1 is the control.

Enzymological study recorded a significantly increased ($P<0.05$) in the activities of GOT and GPT in liver exposed to the experimental diets than that of the control group (Fig. 2 and 3)

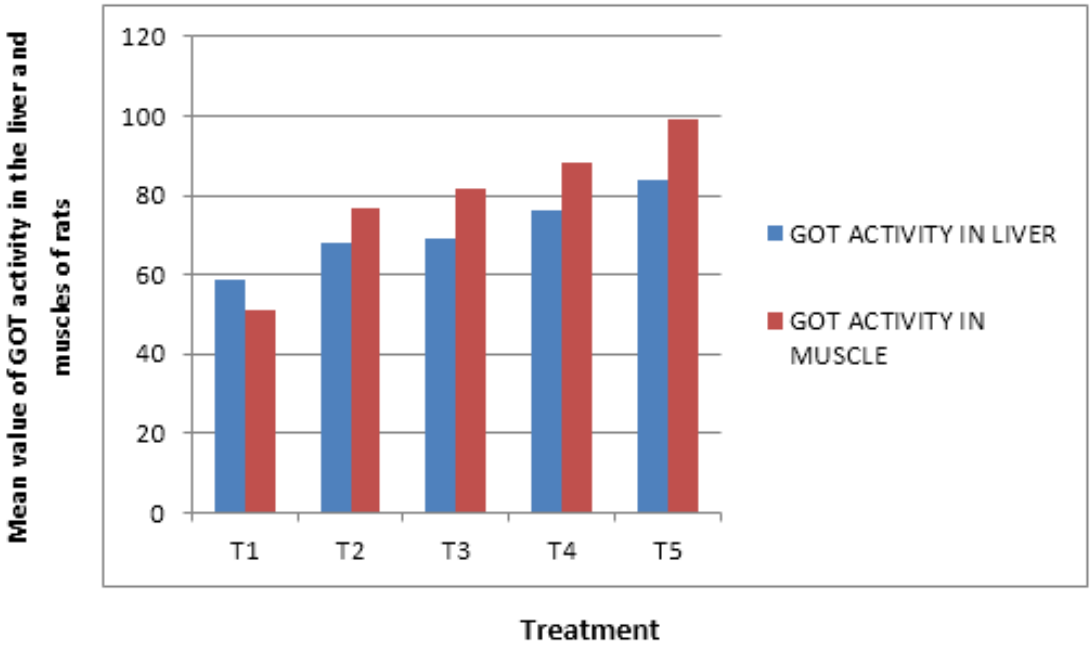

Fig 2: GOT activity in liver and muscle of control and treated rats

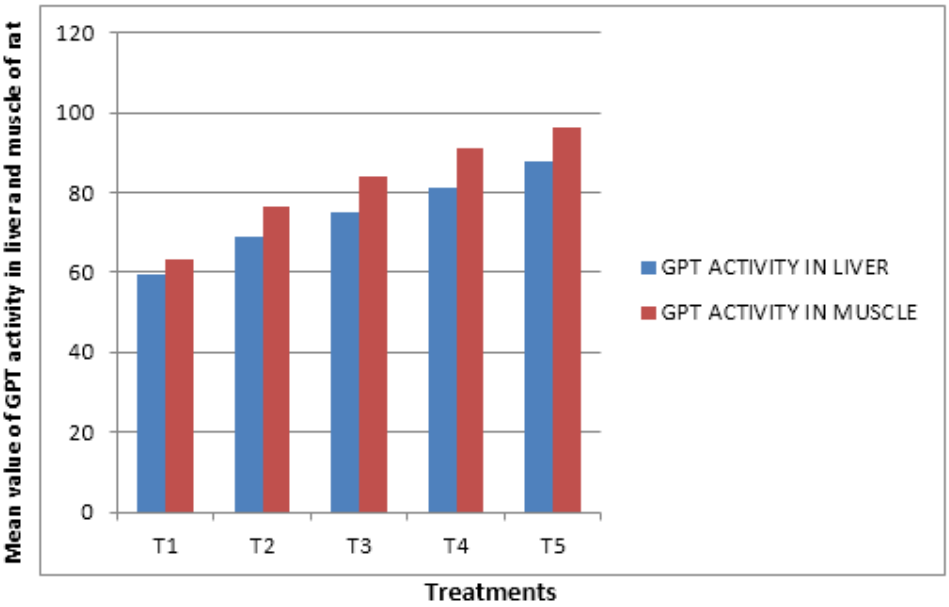

Fig 3: GPT activity in the liver and muscles of control and treated rats.

4. Discussion

The stunted growth and high mortality rate observed among the treated rats may be attributed to the high anti-nutritional contents of the experimental diets. Tannin have been reported to interfere with digestion by displaying anti-trypsin, anti-prolin and anti-amylase activity in animal [18]. Tannin can also form insoluble complexes with proteins thereby reducing the absorption as protein in the system due to phenolic hydroxyl groups which produces unstable radicals [19]. However, high level of tannin in the experimental diet might have form insoluble complexes with the proteins in the diets which are required for growth and well being of animal, thereby reducing its absorption and making the protein unavailable for use. This finding is also in agreement with [20] who reported that tannin causes severe growth depressions in rats. Phytates and Oxalates form insoluble salts with essential minerals like Calcium, Iron, Magnesium and Zinc in food, rendering them unavailable for absorption into the blood as reported by [21]. The same mechanism might be taking place in the rats.

The responses of the blood parameters of the rats to the diets were variable and this is a reflection of the impact of the diets on the different heamatopoetic organs involved in the production of the blood components. Blood parameters are considered patho-physiological indicators of the whole body and therefore, are important in diagnosis the structural and functional status of animals exposed to different diets and toxicants.

Increase in PCV, RBC and Hb of animal is an indicator that the animals are not anemic while decrease level signify anemic [22]. PCV measures the percentage by volume of packed RBC in a whole blood sample after centrifugation [23], it also measures the amount of Haemoglobin (Hb) in grams in 1dL of whole blood and provides an estimate of Oxygen carrying capacity of the RBCs. From this study, there is a significant reduction of PCV, RBCs and consequently Hb of the rats supplied with the experimental diets which may be as a result of the failure to supply the blood circulation with cells from haemohepatic tissue, since the liver has a very important role in the regeneration of erythrocytes and the possible destructive effect on the erythrocyte by toxicant. This result is in agreement with the result obtained by [24] who studied the effect of toxicant on the immune response of rats. Previous haematological studies of nutritional effects brought knowledge that RBCs, PCV and Hb are major and reliable indicators of various sources of stress [25] and these parameters decrease in the presence of anti-nutritional factors [26]. Therefore, a significant increase in the anti-nutritional contents of the experimental diets might had also contributed to the observed decrease in the level of PCV, RBCs and Hb value of the rats supplied with the experimental diets.

WBC count is the number of WBC in a cubic millimeter of whole blood and is usually important in fighting against infections [27]. A significant increase in WBC of rats could be attributed to bacterial infections (stressors) which is foreign to their circulatory system, thereby destroy them [28]. However, the significant increase in WBC count observed among the treated rats may indicate the stress condition of the rats caused by the experimental diets which might have produced hypoxia.

Mean Corpuscular Volume (MCV) measures the average volume of a /red cell in an individual's blood and Mean Corpuscular Haemoglobin (MCH) measures the weight of haemoglobin in a red cell of an individual sample. From the haematological result of this study, the significant decrease in MCV, MCH and MCHC of the rats fed with experimental diets may be reasonably attributed to swelling of red blood cells or realeases of young erythrocytes containing less haemoglobin into the blood circulation as reported by [29]. More so, according to the work of [30], decrease in MCV, MCH and MCHC observed here may be a compensation for impaired oxygen uptake due to lung damage. But, histological examinations on these organs of the treated rats were not carried out in this study to ascertain any damages.

Biochemical parameters can be helpful to identify the target organs of toxic effects and the general health conditions of animals and also, provide early warning of potentially harmful changes in stressed organisms [31]. Increase in blood glucose and decrease in plasma protein level of animals might be as a result of high glucocorticoid which increases the concentration of blood glucose at the expense of protein and fat stores in response to stress [32]. However, similar result was observed. The hyperglycaemic

conditions observed among the treated rats in this study might also be as a result of stress on the rats caused by the experimental diets. [33] reported that animal under stress conditions may mobilize protein to meet energy requirements and to also sustain increased physiological activities. The same mechanisms may also have been operated in this study.

Increased in GOT and GPT indicate hepatic tissue damage [34] . In this present study, the activities of both GOT and GPT increased in the liver and muscle tissue of the treated rats and this may indicate damage of the organs due to accumulation of toxic anti-nutrients in their diets or increased metabolism as the animals tries to mitigate the induced stress. Moreover, detoxification process may not be sufficiently effective to prevent the actions of anti-nutrient in the diets on the system resulting to an increase in GOT and GPT activities in liver and muscle of the rats.

5. Conclusion

The nutritional and toxic effects of Soldier ant meal on Wister Albino rat (*Rattus norvegicus*) has not been studied in details in Nigeria. The finding of this study showed that Soldier ant meal affects the haematological, biochemical and enzymological parameters of the rat negatively. These parameters could be effectively used as potential biomarkers of food toxicity in animals. However, chronic studies on these parameters and the Soldier ant meal need to be further investigated in future

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