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Growth Performance and Hematological Characteristics of Wistar Rats Fed Diets Formulated from Three Species of Mollusks (*Limicolaria flammea*, *Viviparus contectus* and *Egeria radiata*) from Niger Delta Region of Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IF, BAA and MOM wrote the protocol and the first draft of the manuscript. Authors IF and SOG managed the literature searches and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The present study investigated the performance characteristics and hematological parameters of Wistar rats fed diets formulated from three species of mollusks (*Limicolaria flammea, Viviparus contectus* and *Egeria radiata*).

Study Design: Twenty-five Wistar rats (negative control, positive control, 3 experimental groups) were used in the 28-days feeding experiment and administered diets as follows: T_1-Basal, T_2-Cerelac^{TM} (Nestle Foods Nigeria Plc), T_3- *L. flammea*, T_4- *V. contectus* and T_5- *E. radiata*.

Methodology: Proximate composition of diets and faecal and carcass nitrogen were determined by standard methods while hematological analysis was done using Mindray PC-10 Auto Hematology Analyzer.

Results: Crude protein composition of the experimental diets ranged from 6.74±0.03% to 19.58±0.02% in T_1 and T_5 respectively. T_2, T_3, T_4 and T_5 animals had significant weight gain except T_1. T_3, T_4 and T_5 had higher total feed consumed values compared to T_2. There was no significant difference in feed conversion ratio (FCR) between T_2 and experimental diets (T_3, T_4, T_5). T_1 had a negative feed conversion ratio (FCR) (-0.03±0.002) and hence significantly lower ($p<0.05$) than T_2, T_3, T_4 and T_5. Protein efficiency ratio (PER) was lowest in T_5 and highest in cerelac group; Protein efficiency ratio (PER) of T_2 was significantly higher ($p<.05$) than other groups while T_1 was significantly lower ($p<.05$) than other groups. True digestibility (TD) in T_4 was higher than other experimental groups (T_1, T_2, T_3, T_5). Biological value (BV) showed the following trend: $T_5<T_3<T_4<T_2$. There was no significant difference ($p>0.05$) in the relative organ weights of the heart, kidney and spleen of rats in this study. Except for T_3, all other diets boosted packed cell volume (PCV) levels higher than controls (T_1 and T_2). T_4 animals had higher values for packed cell volume (PCV), white blood cells (WBC) and lower values for monocytes and neutrophils when compared to T_1 and T_2.

Conclusion: The formulated diets thus showed good potential to maintain health (except T_3 which showed significant harmful effects on hematological parameters) hence their consumption (after proper cooking) is thus encouraged.

Keywords: Mollusks; hematology; Kjeldahl; proximate; diet.

1. INTRODUCTION

"Nutrition is an indispensable component of existence. Every individual needs to consume food to provide the appropriate energy needed to sustain life's processes of metabolism. Nutrition is defined as the act of nourishing or being nourished" [1]. Ibeh et al. [1] further stated that "nutrition refers to the series of processes by which an organism takes in and assimilates food for promoting growth and replacing worn out or injured tissues". Olusanya [2] defines "food as any substance which after consumption, digestion and absorption by the body produces energy, promotes the growth and repair of tissues and regulates all the body processes". One very indispensable component of food is protein. The level of protein in the food is a function of the developed or developing status of any country. Nigeria, like most developing countries of the world, experiences acute shortage of protein especially protein sources which often results in nutritional problems such as marasmus and protein-energy malnutrition (PEM) [2]. High birth rate has resulted in a population explosion in Nigeria with very minimal growth of the agricultural sector. The importance of food to the continual existence of all humans cannot be over-emphasized. Good health is strictly hinged on good nutrition. Diet refers to the food consumed by a person; it is largely determined by the availability, the processing and palatability of foods. Shellfish has been reported to help lower the risk for heart disease and high blood cholesterol [3]. Although shellfish can be a healthy part of many people's diets, providing significant amounts of high-quality protein, essential nutrients and healthy fats, there are some risks associated with its consumption. Shellfishes are filter-feeders hence water and environmental quality can affect their safety because they can accumulate heavy metals (such as cadmium, Cd; Lead, Pb and Chromium, Cr) which causes disturbance to the metabolic system [3]. Due to the high pollution rates of the Niger Delta region [4], the massive consumption/demand for edible shellfishes, its commercial and industrial importance, this work is undertaken to throw more light on the nutritional relevance of these organisms and their potential toxicological effects on consumers. The safety of foods is a very important factor in determination of the nutritional status of any nation or population. "Food availability and affordability are very important factors that determine the nutritional status of a particular population. A balanced diet which can support optimal growth and development must contain the food nutrients in their appropriate quantities. Protein sources are usually more expensive than carbohydrates hence most indigent families may not be able to provide adequate protein to meet the nutritional requirements of the home. Beef, pork, poultry, mutton and fish are the most common protein sources in Nigeria and they are relatively expensive. Unconventional protein sources such as snails are increasingly becoming popular due to their nutritional benefits. In the Niger Delta region of Nigeria, there are several species of mollusks (aquatic and terrestrial) which are easily accessible sources of proteins to the teeming populations along the coastline. Snail meat is commonly referred to as “Congo meat” and is consumed mostly by people living in the oil-rich Niger Delta region. Snail meat is considered to be very nutritive and rich especially in protein. Several species of snails especially (*Archatina* and *Archachatina*) are widely consumed as meat in Nigeria, however certain species such as *Limicolaria flammea* and *Viviparus contectus* are generally less consumed. *L. flammea* and *V. contectus* are mostly consumed by the low-income earners especially those residing in the coastline and rainforest areas. In some cases, they actually form the largest single item of animal protein in the diet of the common people in the rural areas” [5]. *Egeria radiata* (freshwater clam) is a very nutritious clam abundant mostly in the rainy season. In recent times, there has been an increase in the consumption of mollusks hence there is the need to investigate these food sources as part of the diet and their role in maintaining and sustaining health. This study was designed to investigate the growth performance of these species of mollusk and their effects on hematological parameters.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The snail species used in feed formulation (*L. flammea, E. radiata* and *V. contectus*) were harvested and/or purchased from Yenagoa town in Bayelsa State of Nigeria between the months of May and September during the peak periods of their availability. After collection, the samples were washed properly with tap water and the soft tissues removed from the shells. The samples were thoroughly washed several times and rinsed with distilled water and dried to constant weight in a laboratory oven (TT- 9023A Techmel & Techmel USA) at 80°C for 72 hours. The samples were then ground to fine powder in an electric mill (Masterchef^{TM}).

2.2 Experimental Design

The method of Onyeike, et al*.* [6] was adopted. A total of twenty-five Wistar rats were used in the feeding experiment that lasted twenty-eight days. The rats were bred at the Animal House of the Department of Biochemistry, University of Port Harcourt, Nigeria. The rats were weighed and acclimatized for four days prior to feeding. The initial weights of the animals were taken after acclimatization. The rats were then allocated on the basis of weight and litter origin to 5 groups (negative control, positive control and 3 experimental groups) of 5 rats each in such a way that the group’s mean weight did not differ by more than ±2.00g/mean rat weight. Each treatment group was assigned one of five diets, composition of which is given in Table 1: T_1-Basal, T_2-Cerelac^{TM} (Nestle Foods Nigeria Plc), T_3, T_4 and T_5 (T_3, T_4 and T_5 diets were formulated at 10% protein). Each rat was housed in a single cage with facilities for food and water given *ad libitum* for 28 days. On a daily basis, the faeces of each rat were pooled, dried in a laboratory oven (TT-9023A Techmel & Techmel USA) at 105ºC for 24 hours and ground into fine powder for faecal nitrogen determination. Data on feed consumption and spilled feed were obtained by recording the amount of feed measured out for each rat at the beginning and the quantity remaining at the end of the experiment. At the end of the feeding period (28 days), the rats were finally weighed and sacrificed (by euthanization) carcass was weighed and appropriately labeled and incisions made into the thoracic and body cavities. Liver, kidney, heart and spleen of each rat was excised, weighed and returned into the individual carcasses and each carcass was oven-dried at 80ºC for 72 hours, ground and stored in a desiccator for nitrogen determination. Faecal and carcass nitrogen were determined by standard methods [7]. Blood samples were also taken from each animal for hematological analysis using Mindray PC-10 auto Hematology analyzer. The methods of AOAC [7] were used for the proximate analyses of the samples. Protein efficiency ratio (PER), Food conversion ratio (FCR), Net protein retention (NPR), Net protein utilization (NPU), True digestibility (TD) and Biological value (BV) were determined by methods described by Amadi [8].

Table 1. Diets formulation

Component (g)	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
Corn flour	690	-	545.3	535.6	514.2
Sucrose	120	-	120	120	120
Vitamin/mineral mix	60	-	60	60	60
Powdered cellulose	50	-	50	50	50
Palm oil	80	-	80	80	80
Cerelac ™	-	1000	-	-	-
Basal	-	-	-	-	-
Limicolaria flammea	-	-	144.7	-	-
Viviparus contectus	-	-	-	154.4	-
Egeria radiata	-	-	-	-	175.8
Total (Kg)	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**

2.3 Statistical Analysis

Data were expressed as means and standard deviations. The data were subjected to analysis of variance (ANOVA) using SPSS and significant differences determined at $p \leq .05$.

3. RESULTS AND DISCUSSION

The proximate composition of experimental diets is shown in Table 2. Crude protein composition of the experimental diets ranged from 6.74±0.03% to 19.58±0.02%, crude fat ranged from 6.08±0.00% to 9.15±0.01%, carbohydrates ranged from 50.85±0.02% to 78.73±0.10%, ash ranged from 2.20±0.20% to 10.60±0.04%, moisture ranged from 2.9±0.01% to 5.82±0.00% while crude fibre ranged from 3.15±0.01% to 8.72±0.04% in all experimental diets. The high carbohydrates composition of the diets may be due to the corn flour and sucrose used in the formulation. The crude protein, crude fat and ash composition of experimental diets were higher than that reported by [9] who formulated experimental diets from African yam bean seeds (*S. stenocarpa*). Amadi [8] reported higher concentrations of crude protein, crude fat, crude fibre and moisture in traditional diets (“Onunu”, “Mgbam”, “Ntit-Ikpa”, “Ntubiri”, “Ji-Otor”, and “Ntubiri-Ikpa”) in his study on nutritional evaluation of selected traditional diets of the Ikwerre people of Niger Delta, Nigeria. The experimental diets thus contained competitive protein levels when compared to diets consumed in some parts of Nigeria [8,10]. Body weight change is a function of feed consumed and the ability to utilize the food [11]. Experimental animals had significant weight gain except basal group (T_1) that had a weight loss (this may not be unconnected with its low protein content). Proteins aid growth and repair of damaged tissues in animals hence poor protein in diets will lead to emaciation [2]. The body weight change of rats on experimental diets (T_3, T_4, T_5) were higher than the positive and negative controls (T_2, T_1). This is an indication that the experimental diets were more palatable thus the rats fed more and consequently had higher weight gains. The body weight change of rats in this study was higher than reports by Ikewuchi, et al*.* [12] on performance characteristics of ungerminated and germinated fluted pumpkin (*Telferia occidentalis*) seed meal on albino rats. With the exception of diet 3 (T_3), the body weight change of rats was higher than reported by Amadi [8]; this is as a result of the higher protein content of the diets in present study. A correlation exists between weight gain and coronary heart disease, obesity, hypertension, diabetes mellitus and arteriosclerosis [2] hence the weight gain of rats on diets T_3 and T_5 can be suggestive of obesity and may not be useful in the management of hypertension, obesity and dyslipidemia. Feed consumption is directly related to feed palatability and experimental animals will consume less of a poor diet. The experimental diets (T_3, T_4, T_5) had higher total feed consumed values when compared to T_2; this is an indication that the experimental diets were more palatable than cerelac (T_2). The total feed consumed in this study is higher than previous reports of feeding studies in rats [9,10,11,13]. There was no significant difference in feed conversion ratio (FCR) between the positive control diet (T_2) and experimental diets (T_3, T_4, T_5); this may be as a result of the protein content of the diets. Basal group (T_1) had a negative FCR (-0.03±0.002) and hence significantly lower ($p<.05.$) than the positive control (T_2) and experimental groups (T_3, T_4, T_5). Protein efficiency ratio (PER) was lowest in diet 3 (T_5) and highest in cerelac group; the cerelac group was significantly higher ($p<.05$) than other groups while basal group was significantly lower (*p<.05*) than other groups. Protein efficiency ratio (PER) values show a direct relationship with body weight gain [8]. Protein efficiency ratio (PER) value below 1.5 indicates a protein of poor quality, 1.5-2.0 intermediate quality and above 2.0 as good quality protein [14]. The findings of this study thus indicates that proteins in the experimental diets (T_3, T_4, T_5) were poor quality and were not properly utilized by the animals. Protein efficiency ratio (PER) values reported in this study were lower than previous reports [8,9,10,11,13]. Net protein retention (NPR) values ranged from of 0.21±0.05 to 1.61±0.34 while net protein utilization ranged from 9.96±4.02 to 59.89±33.10. True digestibility (TD) in T_4 was higher than other experimental groups (T_1, T_2, T_3, T_5). This may be as a result of better digestibility of the animal protein in the experimental diets [15]. The biological value (BV) results in this study agrees with the trend in other studies [11,15]. There was no significant difference (p>.05) in the relative organ weights of the heart, kidney and spleen of rats in this study (Table 4). Hematological characteristics of experimental animals administered the diets is shown in Table 5. Treatment T_3 had the lowest values for PCV, Hb and WBC. With the exception of T_3, all other experimental diets boosted PCV levels higher than the control diets (T_1 and T_2). T_4 animals had higher values for PCV, WBC and lower values for monocytes and neutrophils when compared to the controls (T_1 and T_2). The findings of this study on the hematological parameters of rats fed diet 4 agrees with the findings of Archibong, et al*.* [16] with the exception of WBC, eosinophils, lymphocytes and neutrophils. The findings of this study on the hematological parameters of rats fed experimental diets were slightly lower than those reported by Andrew et al*.* (2018) on Wistar rats fed on diets containing calcium carbide ripened mango fruits and the report of Eissa, et al*.* [17] on biochemical and histopathological studies on female and male Wistar rats fed on genetically modified soybean meals (Roundup Ready).

Table 2. Proximate composition (%) of control and experimental diets (formulated from *L. flammea, V. contectus* and *E. radiata*)

Parameter	T_1	*T_2	T_3	T_4	T_5
Crude protein	$6.74±0.03^{a}$	15.0	$13.47±0.04^{b}$	$17.46±0.10^{c}$	$19.58±0.02^{c}$
Crude fat	$6.08±0.00^{a}$	9.0	$9.15±0.01^{a}$	$7.28±0.00^{a}$	$8.49±0.09^{a}$
Carbohydrates	$78.73±0.10^{b}$	64.2	$56.70±0.00^{a}$	$50.85±0.02^{a}$	$54.60±0.05^{a}$
Ash	$2.20±0.20^{a}$	2.3	$8.35±0.08^{c}$	$9.85±0.19^{c}$	$6.01±0.35^{b}$
Moisture	$3.10±0.06^{b}$	2.5	$4.72±0.20^{b,c}$	$5.82±0.00^{c}$	$2.9±0.01^{a}$
Crude fibre	$3.15±0.01^{a}$	7.0	$7.6±0.21^{c}$	$8.72±0.04^{c}$	$8.42±0.12^{c}$

*Values are expressed as mean ± Standard deviation (n=3); Values in the same row bearing similar superscripts letters are not significantly different at p>.05; * Manufacturer's specification*

Table 3. Performance characteristics of Wistar rats fed control and experimental diets (formulated from *L. flammea, V. contectus* and *E. radiata*)

Parameter	T_1	T_2	T_3	T_4	T_5
BWC(g)	$-4.83±0.26^{a}$	$48.84±22.18^{c}$	$64.70±12.67^{cde}$	$30.74±11.68^{b}$	$64.18±5.61^{cde}$
TFC (g)	$189.43±43.3^{a}$	$212.20±52.2^{a}$	$269.25±13.3^{b}$	$248.23±35.8^{b}$	$268.28±12.6^{b}$
CN(g)	$6.77±1.33^{a}$	$7.14±0.77^{a}$	$7.52±0.90^{a}$	$7.50±0.57^{a}$	$6.79±1.01^{a}$
FN(g)	$3.24±0.62^{a}$	$3.65±0.33^{ab}$	$2.81±0.38^{ac}$	$2.85±0.25^{ac}$	$3.11±0.21^{ac}$
TN(g)	$2.42±0.55^{a}$	$5.09±1.25^{a}$	$29.77±1.47^{bc}$	$25.72±3.71^{cef}$	$24.42±1.15^{bef}$
TP(g)	$15.16±3.46^{a}$	$31.84±7.82^{a}$	$186.70±8.33^{bc}$	$160.73±23.2^{ce}$	$152.59±7.17^{bf}$
FCR	$-0.03±0.002^{a}$	$0.22±0.06^{b}$	$0.24±0.038^{b}$	$0.12±0.34^{b}$	$0.24±0.017^{b}$
PER	$0.04±0.02^{a}$	$1.47±0.38^{c}$	$0.35±0.05^{b}$	$0.19±0.05^{b}$	$0.03±0.01^{a}$
NPR	-	$1.61±0.34^{b}$	$0.37±0.05^{a}$	$0.21±0.05^{a}$	$0.45±0.03^{a}$
NPU (%)	-	$59.89±33.10^{b}$	$11.10±1.97^{a}$	$12.26±1.18^{a}$	$9.96±4.02^{a}$
TD (%)	-	$91.18±7.78^{a}$	$96.57±11.31^{a}$	$101.32±1.09^{b}$	$97.55±6.96^{b}$
BV (%)	-	$65.77±35.56^{b}$	$11.62±2.34^{a}$	$12.11±1.23^{a}$	$10.17±4.01^{a}$

Values are presented as mean ± standard deviation (n=5); Values in the same row with different superscript letters are significantly different (p<.05); BWC-Body weight change; TFC-Total feed consumed; CN carcass nitrogen; FN-Faecal nitrogen; TN-Total nitrogen; TP-Total protein; FCR-Feed conversion ratio; PER-Protein efficiency ratio; NPR-Net protein retention; NPU-Net protein utilization; TD-True digestibility; BV-Biological value

Table 4. Organ weights (g) of Wistar rats fed control and experimental diets (formulated from *L. flammea, V. contectus* and *E. radiata*)

Diet	Liver	Heart	Kidney	Spleen
T_1	$2.03±0.40^a$	$0.20±0.00^a$	$0.37±0.06^a$	$0.27±0.15^a$
T_2	$3.28±1.48^b$	$0.40±0.10^a$	$0.66±0.17^a$	$0.60±0.30^a$
T_3	$4.56±0.58^b$	$0.44±0.11^a$	$0.62±0.15^a$	$0.66±0.11^a$
T_4	$2.62±0.31^a$	$0.88±0.66^a$	$0.68±0.20^a$	$0.48±0.24^a$
T_5	$4.66±0.35^b$	$0.42±0.45^a$	$0.70±0.10^a$	$0.70±0.07^a$

Values are presented as mean ± standard deviation (n=5)
Values in the same column with different superscript letters are significantly different (p<..05)

Table 5. Hematological parameters of Wistar rats fed control and experimental diets (formulated from *L. flammea, V. contectus* and *E. radiata*)

Parameter	T_1	T_2	T_3	T_4	T_5
PCV (%)	$36.00±3.00^a$	$38.60±2.07^{ac}$	$25.40±2.07^{bd}$	$39.80±1.92^{bc}$	$39.00±1.58^{ac}$
Hb(g/dl)	$11.43±1.38^{ac}$	$12.16±0.93^a$	$7.96±0.51^b$	$12.74±0.74^a$	$12.50±0.56^a$
WBC($×10^9$/L)	$4.90±0.26^a$	$6.04±0.55^b$	$3.74±0.30^c$	$6.52±0.36^b$	$3.40±0.22^c$
RBC($×10^{12}$//L)	$5.80±0.56^a$	$6.28±0.32^{ac}$	$3.86±0.27^{bd}$	$6.22±0.29^a$	$6.58±0.35^b$
Platelets($×10^9$/L)	$220±0.03^a$	$298±0.02^c$	$265±0.08^b$	$281±0.06^b$	$294±0.32^c$
Monocytes (%)	$7.00±1.00^a$	$6.00±0.71^{ac}$	$9.60±1.14^{bd}$	$4.40±0.54^{bd}$	$5.60±0.55^{bc}$
Eosinophils (%)	$3.33±0.58^a$	$2.40±0.54^a$	$2.60±0.89^a$	$3.80±0.44^a$	$2.80±0.45^a$
Lymphocytes (%)	$36.00±3.61^a$	$46.20±2.38^b$	$26.20±2.28^b$	$46.40±2.07^b$	$26.60±2.41^b$
Neutrophils (%)	$16.00±2.00^a$	$14.80±1.92^a$	$10.20±1.30^b$	$11.80±1.92^b$	$7.00±1.00^b$

Values are presented as mean ± standard deviation (n=5); Values in the same row with different superscript letters are significantly different at 5% level (p<.05); PCV-Packed cell volume; Hb-Hemoglobin; WBC-White blood cells; RBC-Red blood cells

Basophils were not detected in all the animals used in this study which is in agreement with previous reports [16,18]. The findings of this study on concentration of PCV and Hb are similar to those of Agiang et al*.* [19] however RBC, WBC, lymphocytes and eosinophils were much higher in the present study. Concentrations of hemoglobin (Hb) and PCV in this study corroborates the report of Eyong et al*.* [20] on hematoxic effects following ingestion of Nigerian crude oil and crude oil polluted shellfish by rats; values for WBC and RBC were however much lower compared to those obtained in this study.

4. CONCLUSION

The desire to provide naturally available good quality protein remains a major concern for Nutritionists, Governments and Mothers who manage households. This study has shown that the quality of protein of *L. flammea, V. contectus* and *E. radiata* ensures the maintenance of health while boosting hematological parameter (except *L. flammea*). Significant weight gain was also observed hence these mollusks can be very good for growing children and in the management of protein-energy malnutrition (PEM). However, due to its negative impacts on some hematological indices, consumption of *L. flammea* should be carefully monitored.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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