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Full Length Research Paper

The blood physiology of laboratory mice, *Mus musculus* L. fed with various sources of animal proteins

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Abstract

The laboratory mice, *Mus musculus* L are small mammal of the order Rodentia. They are popular as specimen of scientific research in various fields which include biochemistry, medicine, microbiology and zoology. Forty laboratory mice *M. musculus* of 4 weeks old were used for this experiment. The initial weights of the mice were taken with analytical weighing balance and they were randomly distributed into eight dietary cages A-H and they were fed poultry grower's mash (control diet) at 40% of body weights. The mice in dietary cages D and H were fed the control diet. The mice in dietary cages A-C were given 5g, 10g and15g dosages of dried and pulverized lizard meals plus the control diet while the mice in dietary cages E-G were given 5g, 10g and 15g dosages of dried and pulverized cricket meals plus the control diet. The study lasted for 12 weeks. At the end of the 12th week, the mice were sacrificed to collect their blood for haematological analyses. The blood of each animal was obtained separately and stored in K3EDTA bottles to prevent clotting. Then 0.2 ml of blood was drawn from the mice in each dietary group and a drop of the blood was put on a slide and they were allowed to dry, covered with cover slip and there after viewed under the microscope. The haematological parameters studied included white blood cell, red blood cell, pack cell volume, platelets, hemoglobin, neutrophils, lymphocytes, eosinophils and monocytes. Leucocytes and white blood cells were counted visually; hemoglobin (Hb) concentration was measured by the cyanomethaemoglobin method. Pack cell volume (PCV) was determined by the microhaematocrit technique. Each of the experiment was conducted in triplicate. The results of the haematological analyses revealed that at all dosages lizard meals proved to be better protein supplements than cricket meals because the blood components of the mice improved tremendously in relation to the control animals. The results of this stud show that lizard meals are better than cricket meals, but both are vital sources of protein that could be used in improving the quality of the blood of mice. We conclude that, since haematological parameters reveal vital information about any mammal, the inclusion of quality protein supplements such as cricket and lizard meals in the diets of animals will greatly improve their blood physiology making it able to adequately perform all its functions.

Key words: Detoxify, Erythropoiesis, Haemoglobin, Immunity, Megakaryocytes, Neutrophils, Polycythemia.

Introduction

The laboratory mice *Mus musculus* L. are small mammals of the Order Rodentia which are bred and kept for scientific researches in genetics, physiology, medicine and other scientific disciplines. They are important research tools for modeling human disease progression and development. Haematological and serum biochemical values of the blood of mice have been determined by various authors (Arindkar, Mahesh, Juyal, Majumdar and Perumal (2012); Etim, Williams, Akpabio and Offiong, 2014); Wiedmeyer, Crossland, Veres, Dewey, Felder, Barlow, Vrana and Szalai, 2014). The studies of the haematological parameters of mammals are very important (Omotoso. and Sanya, (2007).

Blood plays important roles in animals among which is the transportation of dissolved gases (Oxygen and Carbon dioxide), transportation of waste products of metabolism (water, urea), hormones, enzymes, nutrients, plasma proteins and blood cells (Fathima and Farhath, 2017). Also, the protection of the body against diseases, regulation of body fluid electrolytes, removal of toxins from the body, maintenance of body temperature, and control of blood pH are part of the functions of the blood. Moreover, the diseases an animal is suffering from can easily be detected if the blood sample is taken and examined (Coffey, Pesavento, Keesler, Singapuri, Watanabe, Watanabe, Yee, Bliss-Moreau, Cruzen, Christe, Reader, von, Gibbons, Allen, Linnen, Gao, Delwart, Simmons, Stone, Lanteri, Bakkour, Busch, Morrison and Van, 2017). Each blood parameters such as the red blood cells (RBC), white blood cells (WBC), platelets, lymphocytes, neutrophils, eosinophils and monocytes has peculiar roles it performs in the blood of animals. It has been established that the diets of animals contribute greatly to the formation and the richness of the blood of the animals (Chen, Shiu, Ho and Jeng, 2017).

The diets of *M. musculus* include cereals, vegetables and fruits. Animal protein is very essential in animal growth and development (Showkat and Neelam, (2017). In constituting feed meals for livestock, fish and beef are always used. However, the high costs of these products have necessitated the need for alternative sources of animal proteins. Lizards are reptiles which belong to the phylum Chordata. The importance of lizards as protein source has been highlighted (Abulude, Adesanya, Ogunkoya, Onibon and Ajayi, 2007). These authors were of the opinion that the protein content of lizard ranged between 54.05 %-57.69 % while the ash content ranged between 12.57% -14.33 % (Abulude et al, 2007). Mineral salts are very important in blood formation in animals. Mineral salt such as iron (Fe) is an important mineral that supports blood formation. Lizard contained 70 mg/kg - 95.9 mg/kg of iron (Abulude *et al*, 2007). This is an indication that lizards are good sources of proteins and mineral salts. Tiamiyu *et al.* (Tiamiyu, Gabriel and Jimoh, 2013) observed that agama lizard meat meal caused weight gains which ranged between 22.85g -42.80g in *Clarias gariepinus* within 8 weeks of treatments. The importance of agama lizards as biomarker of environmental heavy metal pollution has been established (Ogunfowokan, Oyekunle, Akanni and Coker, 2012).

Crickets are arthropods which belong to the Class Insecta and Order Orthoptera. Crickets are pests of crops in warmer climates (Hill, 2008). Insects are very efficient in converting feeds to edible meat (Collavo, Glew, Huang, Chuang, Bosse and Paoletti, 2005); van, 2013); Oonincx, van, van Huis and van Loon, 2015). Apart from being viable and highly nutritious food sources which are rich in protein, vitamins, minerals and healthy fats, insects also produce negligible environmentally harmful greenhouse gases. Insects have been reported to be good sources of proteins, fats, unsaturated fatty acids, minerals, such as iron and zinc, and vitamins, such as thiamin and riboflavin (Kelemu, Niassy, Torto, Fiaboe, Affognon, Tonnang, Maniania and Ekesi, 2015); Rumpold and Schluter, 2013); Paiko, Dauda, Suleiman, Akanga and Jacob, 2013); Agbidye, Ofuya and Akindele, 2009); Ramos-Elorduy, Pino-Moreno and Cuevas-Correa, 1998)). Some insects are also important in healing processes (International Biotherapy Society, 2000). This study was carried out to determine the effects of lizard and cricket meals on the haematological parameters of *M. musculus* mice.

Materials and methods

Collection and preparation of cricket and lizard meal supplements

The dried lizards used for this work and the control feed (vital feed) given to the mice were bought from Oja-Oba in Ado-Ekiti, Ekiti State of Nigeria. The lizards were put inside nylon paper and transported to the Laboratory of Zoology Department, Ekiti State University, Ado-Ekiti, Nigeria. The crickets used were bought from Boode Market in Ibadan, Oyo State of Nigeria. The dried lizards and cricket were further sun-dried for 48 h and then pulverized into fine powders separately with a blender, Qasa model. The pulverized samples were put in separate airtight bowls and kept in the refrigerator at 5^{o} C in the laboratory until required. The samples were kept in the refrigerator to prevent spoilage.

Collection and preparation of Laboratory mice

Forty laboratory mice *Mus musculus* of 4 weeks old were purchased from the Department of Pharmacy, Obafemi Awolowo University, Ile-life, Osun, State of Nigeria. The mice were transported with baskets to the laboratory of Zoology Department, Ekiti State University Ado-Ekiti. The mice were allowed to acclimatize to the laboratory environment for 72 h and their weights were taken with an analytical weighing balance Shanghai Jingtian weighing balance Model JT2101N. The initial weights of the mice ranged from 1.5g to 2.5g. The mice were distributed into the cages that have been designed for the study. A wooden cage with metal gauze made up of 8 chambers was constructed to house the samples during the study period. The cages were labeled A-H dietary groups. Five mice were put in each of the dietary cages. The mice in all the dietary cages (i.e. A-H) were fed with vital feed that constituted 40 % of their body weights. The mice in dietary cages A-C were used to monitor the effects of lizard meals (5g, 10g, 15g lizard meals were added to 40% vital feed respectively), while the mice in dietary cage D were used as the control (they were given only 40% vital feed). The mice in dietary E-G were used to monitor the effects of cricket meals (5g, 10g, 15g cricket meals were added to 40% vital feed respectively) while the mice in dietary cage H were used as control (they were given only 40% vital feed). The mice were fed in the morning and in the evening and the experiments were monitored for 12 weeks.

Haematological analyses

At the end of the twelfth week, the mice were not fed for 24 h before being sacrificed. The mice were kept inside the killing jar containing cotton wool moistened with Chloroform. The blood of the mice in each cage were obtained separately and stored in K3EDTA bottles to prevent clotting (Parasuraman, Raveendran and Kesavan, 2010). The haematological parameters studied included white blood cell, red blood cell, pack cell volume, platelets, haemoglobin, neutrophils, lymphocytes, eosinophils and monocytes (Schalm, Jain and Carrol, 1975). Then 0.2 ml of blood was drawn from the mic in each dietary group and a drop of the blood was put on a slide which has been label 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B, 3C, 4A, 4B, 4C, 5A, 5B, 5C, 6A, 6B, 6C, 7A, 7B, 7C, 8A, 8B, 8C and the slides were allowed to dry. After drying, the slides were stained and covered with cover slip and there after viewed under the microscope. Leucocytes and white blood cells were counted visually; haemoglobin (Hb) concentration was measured by the cyanmethaemoglobin method. Pack cell volume (PCV) was determined by the microhaematocrit technique. Each of the experiment was conducted in triplicate.

Statistical Analysis

The data collected were analyzed by SPSS software and using statistical test. ANOVA followed by the Tukey test for comparing results. A $P<0.05$ was considered as significant.

Results

The result of the effects of lizard meal supplements on the haematological parameters of mice is presented in Table 1. The result showed that lizard meal favoured increase ($p<0.05$) in the Red blood cell (RBC) count of the mice at all treatment dosages. The least ($p<0.05$) value of Red Blood Cell count was obtained in the control. The same trend was observed in the value of platelets present the mice. The platelets content ranged from 341.00±2.01 $x10^5/mm^3$ to 395.50±2.65 $x10^5/mm^3$ while in control the value obtained was 215.00±2.10 $x10^5/mm^3$. Packed cell volume (PCV) in the mice also improved ($p<0.05$) from 7.36±0.05 % to 7.60±0.03 % (from 5g to 15g dosage treatments) in relation to the value obtained in the control (7.25±0.09 %). The haemoglobin content of the blood of the mice in 5g, 10g and 15g lizard meal dosages were higher ($P < 0.05$) than in the control.

The white blood cell contents (WBC) of the blood of the mice were higher ($P < 0.05$) than the value obtained in the mice in the control. The lymphocytes content of the blood of the mice did not differ from the value obtained in the control and the neutrophils content was highest ($P < 0.05$) in the mice in 15g lizard meals dosages than in the mice fed 5g and 10g lizard meal dosages and in the mice in the control. Eosinophils and monocytes content of the blood of the mice followed the same trend observed in neutrophils and other parameters. They were highest ($P < 0.05$) in the mice fed 15g lizard meals dosages than in 5g and 10g treatments and in the mice in the control.

Table 1: Effects of dried and pulverized lizard meals treatments on haematological parameters of *M. musculus*

Parameters	Cage A 5g	Cage B 10g	Cage C 15g	Cage D Control
RBC ($x10^6mm^3$)	8.75±1.00^a	10.65±0.90^b	13.95±1.01^c	3.65±0.25
WBC ($x10^3/mm^3$)	7.15±0.56^a	7.55±0.35^b	13.35±0.12^c	3.85±0.22
Platelets ($x10^5/mm^3$)	341.00±2.01^a	361.00±2.00^b	395.50±2.65^c	215.00±2.10
PCV (%)	7.36±0.05^a	7.45±0.20^b	7.60±0.03^c	7.25±0.09
Hb (g/ dL^{-1})	12. 30±0.13^a	12.50±0.20^b	15.10±0.10^c	11.00±0.17
Lymphocytes (%)	61.00±0.42	61.50±0.31	63.00±0.65	56.00±0.20
Neutrophils (%)	24.50±1.50^a	28.50±0.90^b	30.51±0.52^c	20.50±0.50
Eosinophils (%)	7.00±0.08^a	7.50±0.50^b	8.50±0.50^c	6.00±0.11
Monocytes (%)	3.50±0.10^a	5.50±0.05^a	5.52±0.12^b	2.00±0.01

Each value is a mean of triplicates± Standard deviation of the mean (Tukey test) ($p<0.05$).

Table 2 below showed the result of the effects of cricket meal supplements on the haematological parameters of mice. Red blood cell (RBC) content of the mice fed 15g cricket meal dosage was highest ($p<0.05$) than the values obtained in the mice fed 5g and 10g cricket meal dosages, and the least ($p<0.05$) value was obtained in the control. Platelets, Packed cell volume (PCV), hemoglobin, White blood cells (WBC), lymphocytes, neutrophils, eosinophils and monocytes content of the blood were highest ($p<0.05$) in the mice in 15g cricket meal dosage, followed by the value obtained in 10g cricket treatments and in 5g cricket treatments. The control had the lowest ($p <0.05$) values for all these blood parameters.

Table 2: Effects of cricket meals treatments on haematological parameters of *M. musculus*

Parameters	Cage E 5g	Cage F 10g	Cage G 15g	Cage H Control
RBC ($x10^6 mm^3$)	5.80±0.11[a]	7.95±0.15[b]	10.30±0.10[c]	2.65±0.55
WBC ($x10^3/mm^3$)	6.45±0.15[a]	7.00±0.50[b]	10.30±0.12[c]	3.60±0.10
Platelets ($x10^5/mm^3$)	330.20±2.11[a]	350.02±2.02[b]	379.00±2.29[c]	225.10±2.50
PCV (%)	7.15±0.12[a]	7.30±0.20[b]	7.35±0.10[c]	6.75±0.25
Hb (g/dL^{-1})	11.57±0.11[a]	12.12±0.22[b]	13.31±0.10[c]	9.35±0.21
Lymphocytes (%)	60.50±0.15[a]	61.00±0.11[b]	62.50±0.56[c]	53.50±0.50
Neutrophils (%)	23.50±0.12[a]	24.00±0.21[b]	28.00±0.34[c]	22.00±0.16
Eosinophils (%)	5.50±0.08[a]	6.51±0.10[b]	7.50±0.11[c]	3.50±0.12
Monocytes (%)	3.00±0.05[a]	3.00±0.12[a]	3.50±0.09[b]	2.50±0.10

Each value is a mean of triplicates± Standard deviation of the mean (Tukey test) ($p<0.05$).

Discussion

The blood is a connective tissue which contains formed elements such as erythrocytes, leucocytes, platelets which are suspended and carried in the fluid called plasma (Fathima and Farhath, 2017). The blood serves numerous functions which include the transport of respiratory gases, nutritive molecules, metabolic wastes and hormones (Fathima and Farhath, 2017). In this study it was observed that all the mice fed lizard meals recorded significant improvements in their haematological parameters. These results showed that lizard meal is a good source of proteins thus; corroborating the findings of some authors (Abulude et al, 2007); Tiamiyu et al, 2013); Tiamiyu, Okomoda and Oko, 2014). The reason for the best results of the mice fed with lizard was because its protein content was higher than the value observed in cricket.. Though, some insects have higher percentages of proteins (Omotoso and Sanya, 2007); Ogunleye and Omotoso, 2005); Omotoso, 2015)[a]; Omotoso, 2015)[b]), the red blood cell count of the mice fed lizard meals were consistently higher than the values obtained in three trains of mice (Schnell, Hardy, Hawley, Propert and Wilson, (2002); Spinelli, Motta, José and Godoy, 2014), while the values obtained in the mice fed cricket meal compared favourably with the values obtained by others authors (Schnell, Hardy, Hawley, Propert and Wilson, 2002); Spinelli, Motta, José and Godoy, (2014). However, other factors that could influence haematological parameters of farm animals have been grouped under genetic and non-genetic factors (Agaie and Uko, 1998); Xie, Xu, Liu, Ji, Zhou and Xie, 2013).

The haemoglobin contents of the mice in this study compared favorably with the values reported by some authors (Schnell, Hardy, Hawley, Propert, and Wilson, 2002; Spinelli, Motta, José and Godoy, 2014; RAR, 2009). The richness of the protein supplements might have accounted for the higher contents of hemoglobin obtained in the mice. Packed cell volume has been reported to be involved in the transportation of oxygen and absorbed nutrients (Etim, 2014).The packed cell volume (PCV) of the mice in this study were lower than the standard range (RAR, 2009). An increase in erythrocytes, hemoglobin and packed cell volume is suggestive of polycythemia and positive erythropoiesis (Iranloye, 2002; Mansi and Lahham, 2008; Kuppast, I. J., Vasudeva, N. P. Ravi, M. C. and Birada, 2009; Okpuzor, Ogbunugafor and Kareem, 2009). Erythropoiesis is an increase in the hemoglobin concentration, hematocrit level and the red blood cell count (Raven and Johnson, 2002). Erythropoiesis favours an increase in the oxygen carrying capacity of the blood. Erythropoiesis and hemoglobin synthesis require adequate supplies of vitamins B12, folic acid and mineral iron. If the supply of iron is inadequate, hemoglobin synthesis will be restricted. Raven and Johnson (Raven and Johnson, 2002) observed that most of the oxygen in the blood is present in the erythrocytes where they are chemically bonded to hemoglobin. Each haemoglobin molecule consists of four polypeptide chains called globins and four nitrogen-containing disc-shaped organic pigment molecules called hemes. Normal heme contains iron which helps in gas transport (Raven and Johnson, 2002).

The WBC (leucocytes) of the mice fed with both lizard and cricket meals were higher than the value obtained by (Schnell *et al*, 2002) but fall within the standard range (RAR, 2009). There several kinds of WBC but they all work for the defense of the body. The components of WBC observed in this study include lymphocytes, leucocytes, neutrophils, eosinophils and monocytes while basophil was not detected. The values of the lymphocytes, eosinophils and monocytes obtained in the mice fed cricket and lizard meals fall within the standard range (RAR, 2009) but higher than the values obtained in three strained of mice (Spinelli et al, 2004). Higher lymphocytes range of 76%-84% has been obtained in mice (Schnell *et al,* (2002). Lymphocytes are the only non-phagocytic white blood cells which are important in cellular immune response and antibody production in the body (Raven and Johnson, 2002). Eosinophils are phagocytic and are particularly involved in the destruction of parasitic worms and may also contribute to allergic responses. They also help to detoxify foreign substances, secretes enzymes that dissolve clots and fight parasitic infections (Raven and Johnson, 2002). However, neutrophils and monocytes of the mice in this study compared favorably with those of the three strains of mice (Spinelli *et al*, 2014). In conformity with the results of this study, basophil was not also detected in the mice worked with by some authors (Schnell *et al,* 2002; Spinelli et al, 2014). Neutrophils are highly mobile and they engulf debris or foreign organisms through phagocytosis and they are for immune defenses. Monocytes have the greatest phagocytic potentials of all

body cells and are for immune surveillance. The high content of leucocytes obtained in the mice in this study is an indication of an increase in the immune system of the mice. This result shows that the protein supplements are good in boosting the immunity of the mice against any foreign matter that may invade the body.

The platelets content of the mice fed lizard meals fall within the standard range (Omotoso, 2015) while the platelets content of the mice fed cricket meals were higher than the standard range (Omotoso, 2015). Cricket meal has contributed immensely to the production of more platelets in the mice. Platelets are fragments of large cells called megakaryocytes which are found in the bone marrows of mammals (Raven and Johnson, 2002). The quality and the quantity of blood are influenced by many factors which include breed, age, sex, feeding and environmental conditions (Etim *et al,* (2014); Chinneke, Ologun and Ikeobi, 2006); Melillo, 2007); Jeklova, Leva, Knotigova Faldyna, 2009); Abdel-Azeem, Abdel-Azim, Darwish and Omar, 2010). The lizard and the cricket supplements that were added to the mice feeds actually imparted positively on the quality of the blood of the mice. This finding further justified the observations of Tras, Inal, Bas, Altunok, Elmas and Yazar, 2000) that continuous supplementation of nutrients play significant roles in blood profile and the health of animals. Lizard and cricket supplements are good sources of nutrients that can be utilized in rearing mice and improve their blood qualities.

Conclusion

The results of this study show that lizard meals are better than cricket meals, but both are vital sources of protein that could be used in improving the quality of the blood of mice. Since haematological parameters reveal vital information about any mammal, the inclusion of quality protein supplements such as cricket and lizard meals in the diets of animals will greatly improve their blood physiology thus, making it fit to adequately perform all its functions..

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