HAEMATOLOGY AND RELATIVE ORGAN WEIGHTS OF WEANLING WISTAR RATS FED DIFFERENT DIETARY SOURCES OF ANIMAL PROTEIN

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ABSTRACT
Nutritional values of ingested protein could be easily inferred from haematology and ensuing indices of utilisation like the animals' relative weights of organs. Different protein sources will impact on blood cellular indices and weights of organ in varying manners. Thus, the relative quality of the different dietary animal protein sources on haematological indices and relative organ weights of male weanling Wistar rats was assessed in this study. In a completely randomised design, weanling rats (n=25) were randomly assigned to one of the five dietary treatments: T1-Casein-based diet, T2-Nitrogen-free diet (NFD), T3-Beef meal-based diet, T4- Catfish meal-based diet, and T5-Chicken breast meal-based diet. Dietary treatments and water were provided ad libitum to the rats. Blood (3mL) was sampled from each rat using jugular venipuncture into bottles containing EDTA for haematological analysis. At day 21 of feeding, internal organs of the rats were harvested, the relative weights of organ assessed using standard methods. Data on haematology, leucocyte differentials and weights of organ were analysed using descriptive statistics and ANOVA at α0.05. Rats on T5 (36.00%, 11.82g/dL, and 5.75 x106), had significantly (p<0.05) lower packed cell volume, haemoglobin, and red blood cell counts than those on T1 (42.20%, 14.02g/dL and 7.05x106), T3 (40.80%, 13.34g/dL and 6.87 x106) and T4 (41.6%, 13.64g/dL and 6.92 x106), respectively. The relative weights of the heart, spleen, kidneys, and lungs of rats were similar (p<0.05) across the treatments. Higher weight of lung was observed in T1 compared to other treatment groups (p<0.05). Haematology of rats fed chicken breast meal-based diet tended to be most favourable compared to beef meal or catfish meal based, however, there was no indication of the effect of treatments on the rats' relative organ weights.
Keywords: Haematological indices, Packed cell volume, Relative weights of organ, Relative protein quality.

INTRODUCTION

Animal protein is an essential source of nutrient required for growth, development, and maintenance of the body tissues in humans and animals and provides all essential amino acids required for protein synthesis (Chavey *et al.,* 2003). The essential amino acids are those that cannot be sufficiently synthesised in required quantity by the body and must be obtained through the diets. Animal protein are in various foods such as meat, poultry, fish, eggs, and dairy products. These foods are rich sources of high-quality protein that are easily digestible and absorbable by the body (Mariotti, 2017). The quality of animal protein is determined by its amino acid composition, digestibility, and bioavailability (Phillips and Van Loon, 2011). Different animal protein sources vary in their amino acid composition and quality, depending on the animal species, breed, age, and diet (Elango and Ball, 2016).

Haematology and organ weights are two important parameters that are commonly used in assessing the nutritional status and overall health of an organism (Voslarova *et al.,* 2015). Haematological parameters include the analysis of blood cellular components, such as red blood cells (RBC), white blood cells (WBC), and platelets, while organ weights are useful indicators of the relative size and weights of specific organs in the body which are also reflections of nutrient utilisation. These parameters provide important information about animal's physiological and nutritional state, and are useful indices of deficiencies or imbalances in the diet (Jain, 1993).

Beef meal, chicken breast meal, and catfish meal are animal protein-based diets commonly used in animal feed formulations (Adeyemi *et al.,* 2021). Beef meal is a high-quality protein source derived from beef tissues such as muscle, bone, and cartilage. It is rich in essential amino acids, particularly, lysine, and has a high biological value, making it a valuable ingredient in animal feed formulations. (Perez-Maldonado and Norton, 2006). Chicken breast meal is a high-quality protein source derived from chicken breast meat. It is rich in essential amino acids, particularly leucine, and has a high digestibility, making it an excellent ingredient in animal feed formulations (Adedokun *et al.,* 2017). Catfish meal is a protein source derived from the processing of catfish; a freshwater fish commonly found in the southern United States. It is rich in essential amino acids, particularly methionine, and has a high digestibility, making it a valuable ingredient in animal feed formulations. (Smith *et al.,* 2019). Despite the above documented importance of animal protein in the diets of weanling rats (Odeyemi and Bradley, 2017), little is known about the relative nutritional quality of diets based on these animal protein-ingredients. Therefore, the relative quality of diets based on beef meal, chicken breast meal, and catfish meal on haematology and organ weights of weanling Wistar rats were assessed in this study.

MATERIALS AND METHODS

Experimental Station

The study was carried out at the Rat House facility within the Department of Animal Science, University of Ibadan, Nigeria located on Latitude 7^{0}26'N and Longitude 3^{0}54'E of the Greenwich Meridian East at an altitude of 200m above sea level. Average temperature range and humidity of the location is 23-24℃ and 60-80%, respectively (SMUI, 2018)

Experimental Design and Diets Layout

Weanling male Wistar rats (n=25), weighing 3.0±1.9g were used for the trial. The rats were allocated to five treatments with five rats per treatment in a completely randomised design.

These test ingredients (beef, catfish, chicken breast) were used to formulate the diets for the rats. Detail of dietary formulation for rats is shown in Table 1. The dietary layout is as described below.

Treatment 1 – Casein (Control) diet
Treatment 2 – Nitrogen free diet
Treatment 3 – Diet containing beef meal
Treatment 4 – Diet containing Catfish meal
Treatment 5 – Diet containing Chicken breast meal

Table 1: Composition (g/100g) of the Experimental Diets Fed to Weanling Wistar Rats

Treatments	T1	T2	T3	T4	T5
	Casein diet	Nitrogen free diet	Beef meal	Catfish meal	Chicken breast meal
Casein	12.5	0.0	0.0	0.0	0.0
Cornstarch	69.0	81.5	70.7	70.6	70.6
Cellulose	5.0	5.0	5.0	5.0	5.0
Soya oil	5.0	5.0	5.0	5.0	5.0
Sucrose	5.0	5.0	5.0	5.0	5.0
Table salt	0.3	0.3	0.3	0.3	0.3
Calcium phosphate	2.5	2.5	2.5	2.5	2.5
Limestone	0.5	0.5	0.5	0.5	0.5
Beef meal	0.0	0.0	10.8	0.0	0.0
Catfish meal	0.0	0.0	0.0	10.9	0.0
Chicken breast meal	0.0	0.0	0.0	0.0	10.9
Vit.-Min. premix	0.2	0.2	0.2	0.2	0.2
Total	**100**	**100**	**100**	**100**	**100**
Calculated Nutrient					
Crude protein	11.802	0.652	10.3048	10.4455	11.1678
Energy	364.27	404.02	388.036	393.229	391.158
Fat	5.0278	5.0328	5.02848	5.02844	5.02844
Crude fibre	0.4638	0.4663	0.46414	0.46412	0.46412
Calcium	0.8772	0.9022	0.8806	0.8804	0.8804
Phosphorus	0.69565	0.6144	0.5928	0.5926	0.5926
Non phytate P	0.58775	0.5315	0.5207	0.5206	0.5206

.Min. Premix= Vitamin Mineral Premix. Non phytate P= Non phytate Phosphate-Vit.T1= Casein-based diet, T2= Nitrogen-free diet (NFD), T3= Beef meal-based diet, T4= Catfish meal-based diet, and T5= Chicken breast meal-based diet.

Statistical Analysis
Data were subjected to descriptive statistics and analysis of variance using the general linear model of SAS (2012). Means were separated using New Duncan's multiple range test of the same software at $\alpha_{0.05}$.

RESULTS AND DISCUSSION
Results showed that PCV values were significantly lower (p<0.05) in T2 and T5, compared to other treatments (Table 2). This could be attributed to lack of dietary protein, as it plays a crucial role in erythropoiesis, the process of RBC formation, and haemoglobin synthesis (Wu, 2017). Haemoglobin is an important proteinous cellular pigment found in the red blood cells that carries oxygen throughout the body. The level of Hb in the blood can be affected by various factors, including diet. The T1, T3, and T4 diets may have provided the animals with a higher quality protein important for the production of Hb. On the other hand, T2 was a nitrogen-free diet, which may have contributed to the lower Hb concentrations due to the lack of essential amino acids necessary for Hb synthesis. The T5 was a chicken breast meal diet, which may have provided insufficient amounts of essential nutrients compared to the other diets, resulting in lower Hb concentrations.
The T2 and T5 may be relatively deficient in nutrients essential for RBC production, leading to lower RBC count. One possible explanation for the lower RBC count in T2 and T5 could be the lack of nitrogen in T2, as nitrogen is a key component in the production of haemoglobin, which is present in RBCs. White blood cells (WBCs), also known as leukocytes, are important component of the immune system and helps defend the body against infection and disease. The normal range for WBC counts could vary depending on factors such as age, sex, and health status, but generally falls between 4,500 to 11,000 cells/µL of blood (Mayo Clinic, 2022).
The platelet count was significantly lower in T5 compared to T1 and T3, an indication that T1 and T3 impacted positively on platelet count. The lymphocytes in T1 (72.40%) were higher than in other dietary treatments.
It has been reported that diets deficient in specific nutrients, such as protein, reduces lymphocyte counts in the blood (Turchanowa *et al.,* 2001). Thus, higher MCV in T5 could be due to higher fat content in the diet, as previously reported that high-fat diets could influence red blood cell size (Chavey *et al.,* 2003). The lower liver weights observed in T2 and T4 (Table 3) indicates that these diets may have less of an impact on the liver's metabolic load compared to the casein diet (T1). Previous studies have shown that dietary protein sources could affect the inflammatory response in the lungs (Trivedi *et al.,* 2016; Kim *et al.,* 2011). Overall findings implied that protein sources in the diets could affect the lung weight in rats, possibly through inflammatory response in the lungs.

Table 2: Haematology of male Wistar Rats Fed Different Dietary Sources of Animal Protein

Parameters	T1	T2	T3	T4	T5	SEM	P value
PCV (%)	42.20^{a}	36.20^{b}	40.80^{a}	41.60^{a}	36.00^{b}	0.79	0.01
Hb (g/dL)	14.02^{a}	11.82^{b}	13.34^{a}	13.64^{a}	11.82^{b}	0.27	0.01
RBC (x 10^6)	7.05^{a}	6.08^{b}	6.87^{a}	6.92^{a}	5.75^{b}	0.14	0.002
WBC (x 10^3)	2.86^{b}	3.93^{a}	2.92^{b}	2.88^{b}	3.31^{ab}	0.14	0.05
Platelets (x10^4)	8.76^{a}	7.70^{ab}	8.40^{a}	5.90^{ab}	4.68^{b}	0.56	0.09
Lymphocytes (%)	72.40^{a}	66.40^{b}	69.40^{ab}	70.20^{ab}	65.00^{b}	0.93	0.07
Neutrophils (%)	24.20^{b}	28.20^{ab}	26.00^{ab}	27.00^{ab}	31.80^{a}	0.97	0.13
Monocytes (%)	1.40	1.80	2.20	1.20	1.80	0.16	0.33
Eosinophils (%)	2.00	1.60	2.40	1.60	1.40	0.26	0.78
MCV	59.85^{b}	59.59^{b}	59.42^{b}	60.13^{b}	62.63^{a}	0.37	0.02
MCH	19.89^{ab}	19.45^{b}	19.42^{b}	19.71^{b}	20.56^{a}	0.13	0.02
MCHC	33.25	32.64	32.67	32.79	32.83	0.11	0.45
N: L	0.34^{b}	0.42^{ab}	0.338^{ab}	0.39^{ab}	0.49^{a}	0.02	0.12

abc Means of treatments along a row with different superscripts differed significantly (p<0.05).

SEM= Standard error of mean. PCV= Packed Cell Volume, Hb= Haemoglobin, RBC= Red Blood Cell, WBC= White Blood Cell, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, N:L= Neutrophils to Lymphocytes ratio, T1= Casein-based diet, T2= Nitrogen-free diet (NFD), T3= Beef meal-based diet, T4= Catfish meal-based diet, and T5= Chicken breast meal-based diet.

Table 3: Relative Organs Weights of Wister Rats Fed on Different Protein Source Diets

Treatments	Liver (%)	Heart (%)	Spleen %)	Kidney (%)	Lungs (%)
T1	4.17^{a}	0.61	0.28	0.94	0.88
T2	3.43^{b}	0.77	0.41	1.06	1.07
T3	3.74^{ab}	0.75	0.39	0.91	0.92
T4	3.44^{b}	0.62	0.31	0.99	0.89
T5	3.90^{ab}	0.74	0.28	1.02	1.06
SEM	0.08	0.02	0.02	0.02	0.04
P value	0.016	0.14	0.11	0.22	0.47

[abcd] Means of treatments along a row with different superscripts differed significantly ($p<0.05$).
SEM= Standard error of mean. T1= Casein-based diet, T2= Nitrogen-free diet (NFD), T3= Beef meal-based diet, T4= Catfish meal-based diet, and T5= Chicken breast meal-based diet.

CONCLUSION

Gross haematology of rats fed chicken breast meal-based diet tended to be most favourable compared to beef meal or catfish meal based diets. However, aside from the weight of the liver, there was no indication of the effects of treatments on the rats' relative organ weights.

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