HAEMATOLOGY AND INTERNAL ORGAN WEIGHTS OF MALE WEANLING WISTAR RATS FED DIFFERENT DIETARY PROTEIN SOURCES

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ABSTRACT
Male weanling Wistar rats (n=25) weighing 30-40g were randomly allocated to five dietary treatments in a completely randomised design to assess the haematology and organ weight changes on diets containing Casein-based diet (T1), Nitrogen-free diet (T2), Fish meal-based diet (T3), Soyabean meal-based diet (T4) and Groundnut cake-based diet (T5) for 15 days. At day 15, blood (2.5mL) was sampled from each rats (five/treatment) into a bottle with EDTA for the haematology. All the rats were thereafter sacrificed and dissected. The heart, liver, kidney, lungs and spleen removed and weighed separately. Data were analysed using descriptive statistics and ANOVA at α0.05. Respective packed cell volume (PCV) and haemoglobin in T1 (42.0% and 13.76g/dL) and T3 (42.4% and 14.04g/dL) were significantly higher (p<0.05) than in other treatments. Erythrocytes was highest (p<0.05) in T3 (7.1 x106/mm3) and lowest in T2 (5.8 x x106/mm3) (p<0.05). However, leucocytes, monocytes, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were not significantly affected (p>0.0) by dietary treatments. The liver and spleen weights showed no significantly differences (p>0.05) across treatments. The relative heart weight (%) in T3 (0.46), T4 (0.44), T5 (0.49) were similar but significantly lower (p<0.05) than in T2 (0.51). Rats on T2 had higher kidney weight (1.19) while T1 (0.97), T3 (0.86), T4 (0.85) and T5 (1.00) were similar. Rats fed fish meal based diet showed slight improvement in haematological parameters compared to soyabean meal and groundnut cake. Therefore, dietary fishmeal was considered to be most favourable for the rat haematology. There was no visible effect of the dietary test samples on the assessed organs.
Keywords: Haematological parameters, Relative organ weights, Weanling wistar rats

INTRODUCTION

Protein malnutrition is an important deficiency condition which often occurs during the critical transitional phase of weaning animals, affecting their growth and development. Inadequacies in protein quality will inevitably affect growth, therefore the production of highly nutritious feed rich in protein are made to improve health and nutritional status of animals. (Dahiya and Kapoor, 1993; Gilbert *et al.,* 2011). The major plant protein source is soyabean meal which is more expensive than groundnut cake- another alternative plant protein source, while Fish meal is a major source of animal protein (Adeyemo *et al.*, 2017).

Blood is the major transport system of the body and its constituents change in relation to the physiological conditions of the animals (Ihedioha *et al.,* 2004). Haematological parameters are regarded as valuable tools for assessing animal health and are reported to be affected by dietary protein. (Ferguson *et al*., 2010).

Previous studies have shown the effects of protein quality in complementary diets on the haematological indices and relative organ weights in rats (Rubio *et al.*, 1999; Oluseye *et al.,* 2009; Oluwole and Oluremi, 2012; Adeniyi *et al*., 2018; Christopher *et al.,* 2020). Recent documentation in these regards are imperative to update knowledge on the ingredient nutritive values as well as to compare their relative effects of their metabolism in the animals

Thus, the study was aimed at assessing the haematological parameters and internal organ weight changes of male weanling Wistar rats fed diets containing different protein sources.

MATERIALS AND METHODS

Experimental site

The study was carried out at the Rat House, Department of Animal Science, University of Ibadan, Ibadan, Nigeria. The study area lies between longitude $7^0$27.05 north and $3^0$53.74 of the Greenwich

Meridian east at an altitude of 200m above sea level. Average temperature range and humidity of the location is 23-42^{0C} and 60-80%, respectively (SMUI, 2018).

Experimental design and animal management

Weanling Wistar rats (n=25) weighing between 30 and 40g were purchased from the Department of Anatomy, University of Ibadan. The rats were randomly allocated to five treatments, each replicated five times. Each rat was housed individually in well ventilated stainless-steel metabolic cubicles. The design of the experiment was a completely randomised design.

Experimental diets

Five Diets were formulated as presented in Table 1. The dietary layout is as described below.

Treatment 1- Casein based diet
Treatment 2- Nitrogen-free diet (NFD)
Treatment 3- Fish meal based diet
Treatment 4- Soybean meal based diet
Treatment 5- Groundnut cake based diet

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

Treatments	T1	T2	T3	T4	T5
Casein	12.50	0.00	0.00	0.00	0.00
Cornstarch	69.00	81.50	66.79	58.78	59.28
Cellulose	5.00	5.00	5.00	5.00	5.00
Soya oil	5.00	5.00	5.00	5.00	5.00
Sucrose	5.00	5.00	5.00	5.00	5.00
Table salt	0.30	0.30	0.30	0.30	0.30
Dicalcium Phosphate	2.50	2.50	2.50	2.50	2.50
Limestone	0.50	0.50	0.50	0.50	0.50
Fishmeal	0.00	0.00	14.71	0.00	0.00
Soyabean meal	0.00	0.00	0.00	22.73	0.00
Groundnut cake	0.00	0.00	0.00	0.00	22.22
Vit-Min premix	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100
Calculated nutrients					
Crude Protein	11.802	0.652	11.1697	11.3806	10.6954
Energy	364.27	404.02	389.457	359.738	348.914
Fat	5.0278	5.0328	5.02692	5.81926	5.91271
Crude Fiber	0.4638	0.4663	0.46336	2.05285	1.57286
Calcium	0.8772	0.9022	0.87278	0.92268	0.85776
Phosphorus	0.69565	0.6144	0.58498	0.71671	0.56996
Non Phytate P	0.58775	0.5315	0.51679	0.57015	0.57149

Vit-Min premix- vitamin-mineral premix, T1- Casein based diet; T2- Nitogen free diet; T3- Fishmeal based diet; T4- Soyabean meal based diet; T5- Gruundnut cake based diet

Blood collection and analyses

At day 15 of the experiment, 2.5mL blood was sampled from each rat by jugular venipuncture into bottles containing ethylene diamine tetracetic acid (EDTA) bottles for haematological assessments. Packed cell volume was determined by the micro haematocrit method, haemoglobin concentration was estimated using the cyanmethaemoglobin method (Cannan, 1958) while red blood cell counts and white blood cells counts were determined using a haemocytometer with the improved Neubauer slide (Douglas and Harold, 2004). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from PCV, RBC and Hb using equations from Tazawa *et al.,* 2011. Leucocyte deferential counts (lymphocytes, eosinophils, monocytes and neutrophils) were determined.

Relative Organ weights determination

The liver, kidney, spleen, heart and lungs were harvested from the dissected carcass, and washed in a cold buffer and weighed using a sensitive scale. The relative organ weights was expressed as percent body weight at slaughter that is, organ weights weighed divided by the body weight at day 15, multiply by 100%

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 $\alpha_{0.05}$.

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

Treatments	T1	T2	T3	T4	T5	SEM	P value
PCV (%)	42.00^{a}	35.20^{b}	42.40^{a}	38.60^{ab}	36.00^{b}	0.95	0.03
Hb (g/dL)	13.76^{a}	11.52^{b}	14.04^{a}	12.48^{ab}	11.54^{b}	0.32	0.01
RBC x10^{6}/mm^{3}	6.98^{ab}	5.80^{c}	7.14^{a}	6.32^{ab}	5.92^{b}	0.18	0.04
WBC (x10^{3})	2.71	2.44	2.84	2.30	2.74	0.14	0.78
Platelet (x 10^{4})	20.74^{c}	20.00^{c}	87.00^{a}	80.00^{b}	82.40^{ab}	3.50	0.62
Lymphocytes (%)	72.20^{a}	65.00^{b}	70.20^{a}	68.00^{ab}	65.20^{b}	0.84	0.01
Neutrophils (%)	24.40^{b}	30.60^{a}	27.60^{ab}	26.40^{ab}	30.40^{a}	0.89	0.11
Monocytes (%)	1.40	1.60	2.20	2.00	1.60	0.16	0.19
Eosinophils (%)	2.00^{ab}	2.40^{a}	2.00^{ab}	2.40^{a}	0.80^{b}	0.22	0.10
MCV (%)	60.13	61.31	59.49	61.10	61.02	0.52	0.81
MCH (%)	19.72	20.03	19.71	19.76	19.55	0.14	0.90
MCHC	32.80^{ab}	32.70^{ab}	33.14^{a}	32.34^{b}	32.05^{b}	0.12	0.03
NLR	0.34^{b}	0.48^{a}	0.39^{b}	0.39^{b}	0.47^{a}	0.02	0.05

^{abc} Means of treatments along a row with different superscripts differed significantly ($p<0.05$). PCV= packed cell value, Hb= Haemoglobin, RBC= red blood cell, WBC= white blood cell, MCV= mean corpuscular value, MCH= mean corpuscular heamoglobin, MCHC= mean corpuscular haemoglobin concentration, NLR = Neutrophil to Lymphocyte ratio SEM= standard error of mean; T1= casein based diet; T2= Nitrogen free diet; T3= fishmeal based diet; T4= soyabean meal based diet; T5= groundnut cake based diet.

Table 3: Relative Organ Weights of Experimental Rats Fed Different Dietary Treatments

Treatments	Liver	Heart	Spleen	kidney	Lungs
T1	3.99	0.51^{b}	0.27	0.97^{b}	1.13^{ab}
T2	4.13	0.63^{a}	0.24	1.19^{a}	1.22^{a}
T3	3.60	0.46^{b}	0.31	0.86^{b}	1.08^{b}
T4	3.65	0.44^{b}	0.32	0.85^{b}	0.93^{b}
T5	3.92	0.49^{b}	0.29	1.00^{b}	1.08^{b}
SEM	0.11	0.02	0.01	0.03	0.03
P value	0.52	0.02	0.66	0.00	0.21

^{ab} *Means of treatments along a row with different superscripts differed significantly ($p<0.05$). T1= casein based diet; T2= Nitrogen free diet; T3= fishmeal based diet; T4= soyabean meal based diet; T5= groundnut cake based diet.*

RESULTS AND DISCUSSION

The haematological response of Wistar rats fed different dietary protein sources is shown in Table 2. The PCV was significantly higher ($p<0.05$) for T1 (42.00%) and T3 (42.40%) but lower in T2 (35.20) and T5 (36.00). Significantly higher haemoglobin ($p<0.05$) were in T1 (13.76g/dL) and T3 (14.07g/dL). But lower for T2 (11.52g/dL) and T5 (11.54g/dL). The RBC was highest ($p<0.05$) in T3 (7.14×10^{6}/mm^{3}) but lowest in T2 (5.80×10^{6}/mm^{3}). There was no effect of the treatment on white blood cell ($p>0.05$) with values which ranged from 2.71 to 2.84 (x10^{3}).

Lymphocytes count in T1 and T3 were 72.20% and 70.20%, respectively and were significantly higher ($p<0.05$) than for other dietary treatments. Neutrophils was highest in T2 (30.60) and T5 (30.40) and lowest in T1 (24.40). Platelets in T3 (8.70) and T5 (8.24) were similar and significantly higher ($p<0.05$) than for other treatments. Eosinophile in T4 and T2 (2.40%) and was significantly higher than ($p<0.05$) in other treatments Monocytes, MCV and MCH values were not significantly different ($p>0.05$) across the treatment. However, MCHC was higher ($p<0.05$) in T3 (33.14%) than T3 and T4. Neutrophils to lymphocytes ratio was significantly ($p<0.05$) higher in T2 (0.48), and T5 (0.47) compared to T1 (0.34), T3 (0.39) and T4 (0.39).

The PCV is an index of toxicity, any reduction in its concentration in the blood would suggest usually presence of toxic factors (haemagglutinins) which has adverse effect on blood formation (Oyawole and Ogunkunle, 1998). The balance in the blood components are indications of availability of nutrients for synthesis of blood cells. In the present study, the blood components of rats fed fishmeal

based-diet was better than soyabean and groundnut cake. The observed decrease red blood cell value of rats in T5 could be attributed to the destruction of RBC by the residual anti-nutritional factors in groundnut cake, as it may be deficient in certain essential amino acids, such as lysine and methionine (Olayemi *et al.,* 2015). The present findings agreed with those of Gaines *et al.* (2005) that fish meal of good quality normally contain between 60% and 72% crude protein by weight. Fish meal was reported to contain relatively higher sulphur-amino acids, methionine and lysine, which includes bolsters animals' resistance to numerous diseases and improves growth (Gaines *et al.*, 2005).

The WBC, are part of the immune system that help protect the body from infections, diseases, and foreign substances. Higher WBC count in the blood is frequently a sign of illness. As observed, the present study was consistent with the range reported by Albert (2005).

The NLR is a useful biomarker for accessing inflammation and immune response in various condition including cancer, autoimmune diseases and infections. The NLR is an indicator of chronic stress Melissa and Debra (2014). The result of the study suggest that fishmeal and soyabean meal has better NLR than groundnut cake and an indication of their respective dietary protein quality (Olayemi *et al.,* 2015).

The relative organ weights of experimental rats fed different dietary treatments is shown in Table 3. There were no significant differences ($p>0.05$) the liver and spleen weights across the treatments. The heart weight in T3 (0.46), T4 (0.44), T5 (0.49) and T1 (0.51) were significantly ($p>0.05$) lower than in T2 (0.63). Higher ($p<0.05$) kidney value was in T2 (1.19) than T1 (0.97), T3 (0.86), T4 (0.85) and T5 (1.00). The Lung weight in T2 (1.22) was significantly ($p<0.05$) higher than in T1 (1.13), T3 (1.08) and T5 (1.08)

The relative organ weights of rats were similar for fishmeal, soyabean meal, groundnut cake and casein based-diets except for the lungs. However, the findings corroborates those of Oluseye *et al.* (2009) that relative organ weights were not easily affected by changes in nutrition of animals, and the duration of trial might be too short to cause pronounced effect. Conversely, Adeniyi *et al.* (2018) showed that rats fed lower protein diets had reduced relative organ weights.

In **CONCLUSION**, fishmeal based diet showed slight improvement on haematological parameters when compared to soyabean meal and groundnut cake. There was no visible effect of the dietary test samples on the assessed organs.

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