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Growth Performances of the Laboratory Rats, *Rattus norvegicus* on Various Protein Supplements and the Effects of Some Heavy Metals on the Haematological Analysis of Their Blood

[1]O.T. Omotoso and [2]B.T. Sanya
[1]Department of Zoology, University of Ado-Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria

Abstract: Laboratory rats, *Rattus norvegicus* were fed poultry growers mash plus additional protein supplements and some heavy metals to observe their growth and the haematological parameters of their blood samples. All the dietary supplements resulted in growth increases of the rats. The effects of the diets are significantly correlated at 0.01 levels. Rats recorded weight losses in all heavy metals laced diets except in calcium sulphate laced diets where the rats recorded growth increase. The haematological analysis of the blood of heavy metals-free rats revealed that White Blood Cells (WBC) ranged between 7,800±2.20-17,700±3.10 mm^{-3}, Red Blood Cells (RBC) 4.11±0.50×10^6 mm^{-3} to 6.66±1.82×10^6 mm^{-3}, Packed Cell Volume (PCV) 46.30±0.41-60.51±1.90%. Lymphocytes count ranged between 52.80±0.40×10^9/l- 70.71±0.50×10^9/l while for heavy metals laced experiments, the white blood cells count ranged between 2900±2.06 mm^{-3} to 27,400±6.23/mm^3, Red Blood Cells (RBC) 6.45±0.01×10^6 mm^{-3} to 7.50±0.60×$10^6$$mm^{-3}$, Packed Cell Volume (PCV) 59.00±1.53-71.00±1.32%. Lymphocytes count ranged between 56.00±0.87 ×10^9 /l-71.02±1.02 ×10^9/l. Basophils were not detected in the rats.

Key words: *Rattus norvegicus* , haematology, haemoglobin, lymphocytes, anticoagulant, heavy metals

INTRODUCTION

Food is very important for the development and the survival of all living organisms. Legume seeds and animal proteins are very important sources of proteins and other nutrients for human beings and livestocks (Desphande, 1992; Dixon and Hosking, 1992). The evaluation of protein quality is a key factor in the search for new protein sources as well as in the development of food proteins. These observations had led to the conclusion that to accurately measure the quality of protein, the food intake of experimental group of organisms and the control group should be similar.

Many efforts have been made to fully realize the nutritional potentials of some proteinous foods. Different strategies such as breeding, physical and chemical treatments, germination and fermentation of some vegetable protein sources have been employed (Bau *et al*., 1997). The use of protein concentrates and isolates alone or in combination with other processes which usually involve thermal treatment, has become an important choice in these strategy and has been applied to several legume seeds (Flink and Christiansen, 1993). Studies have been carried out to investigate the effects of modified diets on the life span of the laboratory rat (Dalderup and Keller, 1972). Baba *et al.* (1992) reported a significant reduction in total plasma cholesterol and triacylglyceride levels when rats are fed soyabean. Significant physiological, metabolic and immunological disturbances have been reported in rats as a result of legume consumption (Hajos *et al*., 1995). Earlier studies have strongly suggested that a well controlled nutrition regimen has significantly enhance the growth and development as well as the differentiation and gene expression of mammary tissues of rat (Park *et al*., 1994). Recent studies have shown that heavy metals affect animals in various ways (Mohammed, 1991; Chazaly, 1994). Mercury has been reported to cause significant retardation in the body weight as well as hampering the defence mechanisms of rats, even at lower dosages of 0.1-3 mg (El-Missiry *et al*., 1991, 1992).

Reports on the effects of some proteinous foods and heavy metals on the growth and haematological parameters of the laboratory rats are very scarce, thus, in the present study, attempts have been made to determine the growth responses of the laboratory rats to various: feed supplements; the effects of various feed supplements on the haematological parameters of rats;

Corresponding Author: O.T. Omotoso, Department of Zoology, University of Ado-Ekiti, P.M.B. 5363, Ado-Ekiti, Nigeria

the effects of heavy metals on the growth responses of the laboratory rats and the effects of heavy metals on the haematological parameters of the rats.

MATERIALS AND METHODS

One hundred laboratory rats, *Rattus norvegicus* (50 males and 50 females) of two months old were purchased from the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State of Nigeria. The rats were weighed on an analytical weighing balance before they were distributed randoy into 5 dietary groups labeled A-E. Each group contained 5 males and 5 females. Poultry growers mash (containing 21% protein, 7.2% fat, 4.4% fibre, 5.8% ash) was used as the standard unifying feed and 60% body weights of the rats was given to each dietary groups daily. Dietary group A was the control. Dietary group B, C, D and E have poultry growers mash worth 60% body weights of the rats in addition to 40g of blood meal, fish meal, soyabean meal and prawn meal respectively. Then 500mL of water was given to each dietary group daily and the experiments were monitored for a period of 7 weeks. At the end of every week, the rats in each group were weighed separately. At the end of the seventh week, the final weights of the rats were taken before they were sacrificed. Each of the experiments was conducted in triplicates.

Analysis of haematological parameterset: At the end of the 7th week, the rats were sacrificed and 2.5 of blood was withdrawn from each. Anticoagulant k^+ EDTA (2.0 mg mL^{-1} of blood) was added to the blood to prevent clotting. Leucocytes (WBC) were counted visually and differential WBC was done by the method described by Dascie and Lewis (1975). The Haemaglobin (Hb) concentration was measured by the cyanmethaemoglobin method. The Packed Cell Volume (PCV) was determined by the microhaematocrit technique. Each of the experiment was conducted in triplicates.

Effects of heavy metals-laced diets on the rats: The rats were distributed into 5 dietary groups and fed as explained above. At the end of the 7th week, each of the dietary groups was given 5g of salts weekly except dietary A. the salts used are Silver Nitrate, ($AgNO_3$), Mercury Chloride (HgCl), Lead Chloride ($PbCl_2$), Sodium Chromate ($NaCr_2O_4$) and Calcium Sulphate ($CaSO_4$). The salts were mixed with the water given to the rats. The effects of these salts were monitored for 2 weeks after which their weights were taken. Each of the experiments was conducted in triplicates.

Effects of heavy metals on haematological parameters of the rats: At the end of the second week of metal treatment, the rats were sacrificed and 2.5 mL of blood was withdrawn from each of them using K^+ EDTA (2.0 mg mL^{-1} of blood) as anticoagulated. Further haematological analysis were carried out as explained above. Each of the experiment was conducted in triplicates.

Statistical evaluation: Data are presented as means with their respective standard errors. The relationship between the diets and growth of the rats were determined.

RESULTS AND DISCUSSION

Table 1 shows the results of the effects of various feed supplements on the growth of the rats. All the dietary treatments contributed greatly to growth of the rats. In all the dietary groups, the males recorded more growths than the females except in dietary group D where females recorded higher weights. The rats fed diet E recorded the best growth (20.68g for male and 18.96g for female) and they were closely followed by the rats fed diet D (16.74 g for male and 17.98 g for female) while the least growth was observed in the control (10.04 g for male and 8.23 g for female). The effects of the diets on the rats are significantly correlated (0.01 level) at the seventh week for diet A, fourth week for diet B, 4, 5 and 6th weeks for diet C, 4 and 7th weeks for diet D, 5 and 6th weeks for diet E, respectively.

The results of the various dietary treatments showed that supplementing growers mash with soyabean (D) and prawn (E) gave the best yield. These results disagree with the previous study of Fernandez-Quintela *et al.* (1998). The soyabean seeds were deffated and only 37% of the protein contents of the seeds were made available for the rats. A slight significant increase in the protein contents of the chicks fed soyabean has been reported by Daabees *et al.* (1992). Contrary to the increases in weights observed in all the dietary treatments, weight losses were observed in all the rats given heavy metal-laced diets except the diets laced with calcium sulphate salts (Table 2). Increases in weights were observed in the control (Dietary A) and all the other dietary (B-E) supplemented with calcium sulphate. The greatest weight losses were observed in the diets supplemented with blood meal and mercury chloride. Some of the rats died after consuming mercury-laced diets. All doses of mercury have been reported to cause significant decreases in the water content of the liver and brain of rats (Othman, 1992). There by causing their death. Weight losses of 62.48±2.61 and 57.12±2.34 g were observed in males and females rats in mercury treated diet, respectively Chazaly (1994) reported that within 96 h of exposing *Portunus pelagicus* to mercury, the metal caused

Table 1: Growth responses of the albino rats, *Rattus norvegicus* on additional protein supplements for 7 weeks

	Growers mash (Control) (A) (g)		Growers mash + Blood meal (B) (g)		Growers mash + Fish meal (C) (g)		Growers mash + Soyabean meal (D) (g)		Growers mash + Prawn meal (E) (g)	
The weeks	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	2.10±0.61	0.92±1.17	1.00±0.41	0.60±1.50	1.01±0.24	1.04±1.00	1.91±0.67	1.22±1.13	2.12±1.64	2.10±0.72
2	2.20±1.68	1.56±0.49	2.17±0.03	0.78±0.83	1.30±0.63	2.53±1.47	1.00±0.67	2.35±1.56	3.05±0.92	2.25±0.10
3	0.71±098	1.61±1.02	2.29±0.78	1.45±0.18	1.51±0.91	2.60±1.94	2.0±1.20	2.57±1.91	3.16±0.36	0.85±0.95
4	0.48±0.68	1.32±0.11	1.43±1.02*	1.52±0.97*	2.37±2.76*	2.00±1.63*	2.15±3.07*	2.48±2.21*	2.78±0.95	2.88±0.59
5	0.78±0.79	1.05±0.35	2.02±0.61	1.96±0.99	2.80±2.65*	2.50±2.54*	3.25±2.16	2.22±2.66	1.47±1.79*	2.95±1.84*
6	1.72±0.42	0.35±0.19	1.31±1.17	2.71±1.29	3.44±1.55*	2.26±2.53*	3.41±3.68	3.13±2.78	3.36±2.80*	3.30±0.82*
7	2.05±1.58*	1.48±0.59*	1.83±1.64	2.77±1.47	3.19±1.61	3.80±3.12	3.02±2.25*	4.01±4.08*	4.74±3.40	4.63±0.48
Total weight increase	10.04	8.23	12.05	11.79	15.62	16.73	16.74	17.98	20.68	18.96

Each value is a mean of three values ± Standard error of the mean. The values in the same row followed by * is significantly correlated at 0.01 level

Table 2: Growth responses of the albino rats fed additional protein supplements for 7 weeks and heavy metals for 2 weeks

	Growers mash (Control) (A) (g)		Growers mash + Blood meal (B) (g)		Growers mash + Fish meal (C) (g)		Growers mash + Soyabean meal (D) (g)		Growers mash + Prawn meal (E) (g)	
Heavy metals	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
$AgNO_3$	1.53±0.61	1.17±0.17	-36.50±2.41	-48.79±2.54	-18.76±2.24	-14.58±1.02	-15.45±2.67	-14.27±1.13	-10.92±1.64	-9.75±0.72
$PbCl_2$	1.83±0.68	1.59±0.49	-30.76±2.03	-28.28±1.83	-22.08±1.63	-21.53±1.47	-20.45±2.67	-15.84±1.56	-13.09±0.92	-10.10±2.10
$CaSO_4$	1.34±098	1.20±0.02	1.50±0.21	1.63±0.18	1.79±0.45	1.91±0.29	2.00±0.20	2.04±0.11	2.545±0.12	2.35±0.31
$Na_2Cr_2O_4$	1.93±0.61	2.53±0.61	-5.88±0.44	-6.53±0.66	-8.12±0.76	-5.55±0.51	-8.97±0.89	-10.33±0.41	-4.78±0.11	-3..53±0.61
HgCl	2.83±0.54	2.01±0.65	-57.12±2.34	-62.48±2.61	-55.15±2.22	-50.12±1.68	-57.44±1.23	-53.73±1.98	-45.83±2.09	-55.32±3.01

Each value is a mean of three values ± Standard error of the mean

significant decrease in the biochemical factors of hepatopancreas, particularly lipid which is time dependent. Heavy metals are metals whose densities are greater than 5 gcm^3 and they occur in all environments. The findings of some workers have shown that absorption of heavy metals by organisms can either be by active transport or by passive transport (Chan and Rothstein, 1965; Wedemeyer, 1968). Omotoso (2005) reported that the weights of earthworms increased tremendously when kept in heavy metals laced habitats. Gomaa *et al*. (1995) reported that the accumulation of some heavy metals in fish is a growing threat to human beings due to the increases of industrial water discharge in aquatic systems. Fish type differs in their heavy metals accumulation and their affinity depend on the fish habitats and the type of heavy metal involved. The tolerance of organisms to heavy metals have been reported to vary considerably from species to species (Fayed *et al*., 1992; Zari *et al*., 1994). The rats lost weights because the salts were toxic and could not be regulated within their systems. Moreover, the observed decreases in the weights could also be a confirmation of a decrease in their lipid contents as reported in rats by El-Missiry *et al.* (1991).

Table 3 and 4 show the results of the effects of the various dietary treatments and heavy metals on the haematological parameters of the albino rats. The WBC was higher in the control (female) than the male. This trend was observed in dietary groups C and E. WBC was higher in males in dietary groups B and D. Table 4 also shows that the values of WBC are higher in female rats in the control, dietary groups B, C and D while the value obtained for the male in dietary group E was higher. The rats in dietary group A (control) had the highest WBC (17,700±5.1 mm^{-3}) while the least was recorded in the same group. The trend of WBC is such that females had higher WBC than males in the same dietary group. These trends were reversed in heavy metal-laced experiments (Table 4). Male rats had the highest WBC (27,400±6.23 mm^{-3}) in dietary group A while the least value of 2900 mm^{-3} was recorded for the female rat in dietary group E. The value of RBC was higher in heavy metal-laced diets than in metal free diets. The value of PVC was higher in dietary groups B, D and E while the values obtained for the control and dietary group C are lower than the values obtained for the same dietary group in Table 3. Haemoglobin was higher in heavy metal-laced diets than in metal-free diets. A higher RBC and PCV were observed in the rats in heavy metal-laced experiments. Red blood cells are for the transport of gases (oxygen and carbon dioxide) between the lungs and the cells while white blood cells are for defence. Lower values of PCV have been reported by Umapathy and Reid (1987) in rats. The values of Hb in both experiments compare favourably with each other but are higher than what Umapathy and Reid (1987) reported. Waugh and Grant (2002) reported the haemoglobin count of 13-18 g 100 mL^{-1} and 11.5-16.5 g 100 mL^{-1} for man and woman, respectively. Higher haemoglobin concentration, haematocrit and red blood cell count have been reported in birds and fish,

Table 3: Effect of dietary treatments on the haematological parameters of albino rats fed for 7 weeks

	Growers mash (Control) (A)		Growers mash + Blood meal (B)		Growers mash + Fish meal (C)		Growers mash + Soyabean meal (D)		Growers mash + Prawn meal (E)	
Parameters	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
WBC (/mm^3)	7,800±2.2	17,700±5.1	16,400±5.6	15,600±4.2	10,300±3.1	10,800±5.9	14,800±4.4	9,600±3.5	9,800±2.7	16,500±5.6
RBC (×10^6 mm^3)	6.36±1.40	5.45±2.10	4.11±0.50	6.33±0.91	5.21±0.30	6.66±1.82	6.94±0.50	5.70±0.40	5.79±0.61	5.74±0.32
PCV (%)	59.31±2.00	54.71±1.70	56.40±0.61	56.21±2.63	60.51±1.90	60.50±2.60	59.20±1.70	46.30±0.41	55.11±2.10	49.80±0.60
Hb (g 100mL)	16.30±0.49	16.30±0.49	17.01±0.51	17.30±0.52	18.00±0.54	19.31±0.58	19.01±0.57	15.01±0.45	18.01±0.54	14.80±0.43
Neutrophils (×10^9/L)	18.50±0.44	16.70±0.65	30.03±0.65	28.91±0.20	30.20±1.12	20.80±0.90	24.20±0.25	20.40±0.73	16.03±0.18	34.20±0.51
Lymphocytes (×10^9/l)	70.71±0.50	74.60±1.20	52.80±0.40	57.01±1.60	56.06±1.05	63.50±0.50	59.00±1.60	66.40±0.90	67.80±1.00	53.60±0.40
Monocytes (×10^9/L)	12.40±0.20	10.61±0.20	16.51±0.20	14.30±0.04	12.20±0.02	15.80±0.91	16.20±0.40	14.80±0.30	16.70±0.02	12.10±0.20
Eosinophils (×10^9/l)	-	-	2.10±0.01	1.01±0.00	2.05±0.11	2.01±0.11	1.00±0.00	-	1.00±0.00	1.01±0.03
Basophils (×10^9/l)	-	-	-	-	-	-	-	-	-	-
ESR (mm/h)	02	01	01	00	01	01	01	01	00	00

Each value is a mean of three values ± Standard error of the mean

Table 4: Effect of heavy metal-laced diets on the hematological parameters on albino rats fed heavy metals for 2 weeks

	Growers mash (Control) (A)		Growers mash + Blood meal (B)		Growers mash + Fish meal (C)		Growers mash + Soyabean meal (D)		Growers mash + Prawn meal (E)	
Parameters	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
WBC (/mm^3)	27,400±6.23	10,400±3.50	20,500±5.45	11,500±2.35	14,800±5.01	7,400±2.30	16,100±3.25	9,900±4.5	11,100±3.21	2,900±2.06
RBC (×10^6 mm^3)	6.51±0.12	6.45±0.01	7.50±0.60	6.63±0.24	6.62±0.53	7.32±0.45	6.61±0.10	6.50±0.21	7.30±0.23	6.54±0.01
PCV (%)	59.01±0.78	60.01±1.04	71.00±1.32	61.02±0.87	60.00±0.93	66.05±1.22	66.02±0.53	59.01±1.23	71.01±1.08	59.00±1.53
Hb (g/100 mL)	18.07±0.11	18.00±0.54	20.04±0.64	21.04±0.23	19.04±0.47	21.00±0.13	22.01±0.34	18.07±0.11	25..02±0.02	21.45±0.95
Neutrophils (×10^9/l)	34.00±0.27	24.09±0.19	28.03±0.14	32.05±0.36	24.01±0.52	20.00±0.25	24.02±0.32	32.01±0.43	37.05±0.53	20.02±0.81
Lymphocytes (×10^9/l)	60.01±0.92	65.01±1.44	61.03±1.02	56.00±1.11	65.08±0.98	69.04±1.02	63.01±1.29	56.00±0.875	3.01±0.90	71.02±1.02
Monocytes (×10^9/l)	1.00±0.00	1.01±0.01	1.00±0.00	3.02±0.03	3.05±0.01	1.00±0.00	3.01±0.02	2.00±0.01	2.00±0.02	1.00±0.00
Eosinophils (×10^9/l)	6.00±0.50	10.00±0.12	10.00±0.61	9.05±0.14	8.02±0.18	10.00±0.36	3.00±0.01	2.10±0.00	8.12±0.1 1	8.01±0.00
Basophils (×10^9/l)	-	-	-	-	-	-	-	-	-	-
ESR (mm/h)	01	01	01	01	01	03	00	01	01	00

Each value is a mean of three values ± Standard error of the mean

Clarias lazera by Mohammed (1991) and Mohammed-Assem (1994), respectively. Lymphocytes was higher in female rats in the control, dietary groups B, C and D while the value was higher in male rat in dietary E (Table 3). Female rats had higher lymphocytes value in the control, dietary groups C and E while the males had higher values in dietary groups B and D (Table 4). Monocyte count of heavy metal-free experiments are greater than those of heavy metal-laced experiments. Neutrophils was higher in the male than in the female in the control diet, dietary groups B, C and D while the female rats recorded higher values in dietary group E (Table 3). Neutrophils was higher in the males in the control, dietary groups C and E (Table 4). Neutrophils, lymphocytes, basophils, eosinophils and monocytes are responsible for the defence of the body. Neutrophils are active phagocytes which are developed in the bone marrows and they form the first line of defence against external invasion. The female rats recorded higher lymphocytes values in the control, dietary groups B, C and D while in dietary group E, the male rat recorded a higher value. Eosinophils was not detected in the control diet but the value was higher in male rats in all the dietary groups (Table 3). Table 4 shows that the female rats had higher values of eosinophils in the control and dietary group C while male the rats had higher values in dietary groups B, D and E. The increase observed in the number of eosinophils and lymphocytes are indications of allergic reactions in the body. The metals were treated as foreign bodies and the immune system was increased to take care of the allergic situation.

The lymphocyte/neutrophils ratio in heavy metal-free experiment for dietary group E female (1.56:1) while it is (4.19:1) for male. The values of 5.7:1 and 3.7:1 have been reported by Umapathy and Reid (1987). In heavy metal-laced experiments, the lymphocyte/neutrophil ratio in dietary E are (3.55:1) for female and (1.43:1) for males. The various differences observed in the haematological parameters of the rats could be attributed to the compositions of the diets which are quite different from the conventional rats diets. Dietary habits play an important role in the leucocyte count in humans (Ezeilo, 1974). In general, these experiments have shown that supplementing rats diets with blood meal, fish meal, soyabean meal and prawn meal, which are relatively cheap and are readily available sources of protein, would bring better returns. Based on this study, we suggest that these supplements be added to rats diets.

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