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Growth Performance and Haematology of the Laboratory Rat, *Rattus norvegicus* fed on Protein Supplements and Heavy Metals

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Abstract. Laboratory rat *Rattus norvegicus,* fed on poultry growers mash plus additional protein supplements and some heavy metals, was studied for the growth and the haematological parameters. All the dietary supplements resulted in an increase in the growth of the rats. The rats, fed on growers mash and prawn meal showed the best growth within 7 weeks. Effects of diets were significantly, correlated at 0.01 level. Weight loss was recorded in case of all heavy metal-laced diets, however, calcium sulphate-laced diets resulted in an increase in growth. Mercurous chloride was the most toxic salt which resulted in the greatest weight loss. Haematological analysis of rats revealed that RBCs were higher in the case of heavy metal-laced diets than heavy metal-free diets. Generally, RBC counts were higher in females than in males within a group. Fish meal and prawn meal feeding resulted in higher WBC counts.

Keywords: *Rattus norvegicus,* haematology, heavy metal feed, protein supplements

Introduction

Legume seeds and animal meat are important sources of proteins and other nutrients for humans and livestocks (Omotoso, 2006; Desphande, 1992; Dixon and Hosking, 1992). Evaluation of protein quality is a key factor in the search for new proteinaceous sources as well as in the development of food proteins. These observations have led to the conclusion that for correct determination of the quality of protein, food intake of the experimental and the control groups should be similar.

In order to fully realize the nutritional potentials of some proteinaceous 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; Anderson and Wolf ,1995; Frias *et al.*, 1995). Use of protein concentrates and isolates alone or in combination with other processes which usually involve thermal treatment is an important choice of strategy and has been applied to several legume seeds (Aremu, 1990; Flink and Christiansen, 1973). Several studies have been carried out to investigate the effect of modified diets on the life span of the laboratory rat (Delderup and Keller, 1972; Ross, 1961). Merry and Holehan (1979) reported that dietary energy restriction extends the reproductive life span of female rats while Baba *et al.*, (1992) reported a significant reduction in total plasma cholesterol and triacylglyceride levels when rats were fed on soybean. Significant physiological, metabolic and immunological disturbances have been reported in rats as a result of legume consumption (Hajos *et al.*, 1995). Earlier studies strongly suggested that a well controlled nutrition regimen, significantly enhanced the efficiency of growth and development as well as the differentiation and gene expression of mammary tissues in rat (Park *et al.*, 1994, 1987; Baik 1992a, 1992b). Recent studies have shown that heavy metals affect animals in various ways (Chazaly and Said, 1995; Chazaly, 1994; El-Sayyad and Kholy, 1992; Mohamed, 1991). Mercury has been reported to cause significant decrease in the body weight as well as to hamper the defence mechanism in rats, in dosages as low as 0.1 mg to 3 mg (El-Missiry *et al.*, 1992, 1991).

Reports on the effect of some proteinaceous foods and heavy metals on the growth and haematological parameters of the laboratory rats are very scarce. In the present study, an attempt has been made to determine the effect of various feed supplements and heavy metals, individually, on the growth and the haematological parameters of the laboratory rats.

Materials and Methods

One hundred laboratory rats, *Rattus norvegicus* (50 males and 50 females of two months age) were purchased from the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State of Nigeria, and weighed on an analytical weighing balance. They were then distributed randomly into 5 dietary groups labelled A-E. Each group contained 5 males and 5 females. Poultry growers mash (containing 21% protein, 7.2% fat, 4.4% fibre and 5.8% ash) was used as the standard unifying feed and the feed worth 60% body weight

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of the rat was given to each dietary group daily. Dietary group A was the control. Dietary groups, B, C, D and E were, in addition, fortified with 40 g of blood meal, fish meal, soybean meal and prawn meal, respectively. Besides, daily 500 ml of water was given to each dietary group and the experiments were monitored for a period of 7 weeks. At the end of each week, rats of each group were weighed separately. At the end of the seventh week, final weight of the rats was taken. Each experiment was conducted in triplicate.

Analysis of haematological parameters. At the end of the seventh week, rats were sacrificed and 2.5 ml of blood was withdrawn from each animal. Anticoagulant K^+EDTA (2.0 mg/ml of blood) was added to the blood to prevent clotting. Leucocytes (WBC) were counted visually; differential count was performed by the method described by Dacie and Lewis (1975). Haemaglobin (Hb) concentration was measured by the cyanmethaemoglobin method. Packed cell volume (PCV) was determined by the microhaematocrit technique. Each of the experiment was conducted in triplicate.

Effect of heavy metals on haematological parameters. Rats were distributed into 5 dietary groups and fed as explained above. Each dietary group was weekly given 5 g of salt mixed with water, except group A. The salts used were silver nitrate, ($AgNO_3$), mercurous chloride ($HgCl$), lead chloride ($PbCl_2$), sodium chromate (Na_2CrO_4) and calcium sulphate ($CaSO_4$). Effect of the salts was monitored for 2 weeks after which weight of rats was noted.

At the end of the second week, the rats were sacrificed and 2.5 ml of blood was withdrawn from each of them using K^+EDTA (2.0 mg/ml of blood) as anticoagulant. Further haematolo-gical analysis was carried out as explained above. Each experiment was conducted in triplicate.

Statistical evaluation. Data are presented as mean values with their respective standard errors and relationship between the diets and the growth of the rats has been determined.

Results and Discussion

Table 1 shows the effect of various feed supplements on the growth of rats. All the dietary treatments had substantial contribution in the growth. In all the dietary groups, males recorded more growth than females except the dietary group D, wherein, females recorded higher weights. The rats, fed diet E, recorded the best growth (20.68 g for male and 18.96 g for female); 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). Effect of diets on the rats is significantly correlated (0.01 level) at the seventh week for diet A, fourth week for diet B, fourth, fifth and sixth weeks for diet C, fourth and seventh weeks for diet D and fifth and sixth weeks for diet E.

Results of various dietary treatments showed that supplementing growers mash with soya bean (D) and prawn (E) gave the best yield. These results disagree with the previous study of Fernandez-Quintela *et al.* (1998). In this work, soybean seeds were deffated and only 37% of the protein content of seeds were made available to the rats. Slight increase in the protein content of the soybean-fed chicks has been reported by Daabees *et al.* (1992). Contrary to the increase in weight observed in all the dietary treatments, weight loss was observed in all the rats given heavy metal-laced diets except in those given the diets laced with calcium sulphate (Table 2). Increase in weight was observed in control (A) and in all the other dietary groups (B-E), given feed supplemented with calcium sulphate. The greatest weight loss was observed in the rats fed diets supplemented, with blood meal and mercury chloride. Some of the rats died after consuming mercury chloride-laced diets. All doses of mercury chloride have been reported to cause significant decrease in the water content of the liver and brain of rats (Othman *et al.*, 1993) thereby causing their death. Weight loss of 62.48±2.61 g and 57.12±2.34 g was observed in male and female rats, respectively, fed on mercury chloride treated diet. Chazaly (1994) reported that within 96 h of exposure, mercury chloride caused significant decrease in the biochemical factors of hepatopancreas, particularly lipids, which is time dependent. Heavy metals are metals whose densities are greater than 5 g/cm^3 and they occur in all environments. Findings of some workers have shown that absorption of heavy metals by organisms can be by either active transport or by passive transport (Wedemeyer, 1968; Chan and Rothstein, 1965). Omotoso (2005) reported that the weight of earthworms increased tremendously, when kept in heavy metal-laced habitats. Gomaa *et al.* (1995) reported that the accumulation of some heavy metals in fish, due to the increase in industrial water discharge in aquatic systems is a growing threat to humans. Types of fish differ in heavy metal accumulation and their affinity depends on the fish habitat and the type of heavy metal involved. Tolerance of organisms to heavy metals have been reported to vary considerably from species to species (Omotoso, 2005; Zari *et al.*, 1994; Fayed *et al.*, 1992). Rats lose weight because toxic salts could not be regulated within their system. Moreover, the observed decrease in the weight could also be the confirmation of a decrease in their lipid content as reported in rats by El-Missiry (1991).

Table 3 and 4 show the effect of various dietary treatments and heavy metals on the haematological parameters of the

Table 1. Growth response of the albino rats, *Rattus norvegicus* fed on additional protein supplements within 7 weeks

Weeks	(A)(g) Growers mash (Control)		(B)(g) Growers mash + Blood meal		(C)(g) Growers mash + Fish meal		(D)(g) Growers mash + Soybean meal		(E)(g) Growers mash + Prawn meal	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	11.53±0.61	9.17±1.17	17.59±0.41	18.50±1.50	14.77±0.24	18.00±1.00	19.45±0.67	11.27±1.13	14.92±1.64	12.75±0.72
2	15.83±1.68	11.59±0.49	18.76±0.03	19.28±0.83	17.08±0.63	19.53±1.47	20.45±0.67	14.84±1.56	20.09±0.92	17.10±0.10
3	16.54±098	13.20±1.02	21.05±0.78	20.73±0.18	18.59±0.91	22.13±1.94	22.45±1.20	17.41±1.91	23.25±0.36	17.95±0.95
4	17.02±0.68	14.52±0.11	22.48±1.02*	22.25±0.97*	20.96±2.76*	25.17±1.63*	24.60±3.07*	19.89±2.21*	26.03±0.95	19.83±0.59
5	17.80±0.79	15.57±0.35	24.50±0.61	24.21±0.99	22.76±2.65*	26.67±2.54*	26.85±2.16	22.11±2.66	27.50±1.79*	22.78±1.84*
6	19.52±0.42	15.92±0.19	25.81±1.17	25.92±1.29	26.20±1.55*	27.93±2.53*	30.17±3.68	24.24±2.78	29.86±2.80*	26.08±0.82*
7	21.57±1.58*	17.40±0.59*	27.64±1.64	28.29±1.47	29.39±1.61	30.73±3.12	32.19±2.25*	27.25±4.08*	32.60±3.40	28.71±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 * are significantly correlated at 0.01 level.

Table 2. Growth response of the albino rats, *Rattus norvegicus* fed on additional protein supplements for 7 weeks and heavy metals for 2 weeks

Heavy metals	(A)(g) Growers mash (Control)		(B)(g) Growers mash + Blood meal		(C) (g) Growers mash + Fish meal		(D) (g) Growers mash + Soybean meal		(E) (g) Growers mash + Prawn meal	
	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.

Table 3. Effect of dietary treatments on the haematological parameters of albino rats *Rattus norvegicus* fed for 7 weeks.

Parameters	(A) Growers mash (Control)		(B) Growers mash + Blood meal		(C) Growers mash + Fish meal		(D) Growers mash + Soybean meal		(E) Growers mash + Prawn meal	
	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 (x10^6 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 (x10^9/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 (x10^9/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 (x10^9/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 (x10^9/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 (x10^9/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 of albino rats *Rattus norvegicus* fed heavy metals for 2 weeks.

Parameters	(A) Growers mash (Control)		(B) Growers mash + Blood meal		(C) Growers mash + Fish meal		(D) Growers mash + Soybean meal		(E) Growers mash + Prawn meal	
	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±4.51	11,100±3.21	2,900±2.06
RBC (x10^6 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±1.3
Hb (g/100ml)	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 (x10^9/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 (x10^9/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±0.87	53.01±0.90	71.02±1.02
Monocytes (x10^9/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 (x10^9/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±0.11	8.01±0.00
Basophils (x10^9/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.

albino rats. WBC count was higher in females than in males of the control group and in dietary groups C and E. Whereas, in dietary groups B and D, WBC count was higher in males. Table 4 also shows that the values of WBC count are higher in female rats of the control group and dietary groups B, C, and D while the value for the males in dietary group E was higher. Female rats in dietary group A (control) had the highest number of WBC (17,700±5.1/mm^3) while the least number was recorded in the males of the same group. Generally, females had higher WBC counts than the males of the same dietary group. This trend was reversed in heavy metal-laced feeding experiments of 2 week duration (Table 4). Male rats had the highest WBC count (27,400±6.23/mm^3) in dietary group A, while the least value of 2900/mm^3 was recorded in the female rats of dietary group E. The value of RBC was higher in heavy metal-laced diets than in metal-free diets. The value of packed cell volume (PVC) was higher in dietary groups B, D and E, while the values obtained for the control and dietary group C were lower (Table 3). Haemoglobin was higher in the rats fed on heavy metal-laced diets than those fed on metal-free diets. Higher RBC and PCV were observed in the rats of heavy metal-laced feed experiments. Lower values of PCV have been reported in rats by Umapathy and Reid (1987). The values of Hb in both experiments compared favourably with each other but higher than what Umapathy and Reid (1987) reported. Waugh and Grant (2002) reported haemoglobin, 13-18 g/100 ml and 11.5-16.5 g/100 ml for man and woman, respectively. Higher haemoglobin concentration, haematocrit and red blood cell count have been reported fish, *Clarias lazera* and birds by Mohammed-Assem (1994) and Mohammed (1991), respectively. Lymphocytes were higher in number in female rats of the control group, dietary groups B, C and D, while the value was higher in male rats of dietary group E (Table 3). Female rats had higher lymphocyte values in the control, dietary groups C and E, while the males of the dietary groups B and D had higher values (Table 4). Monocyte counts for heavy metal-free feeds were greater than for heavy metal-laced ones. Neutrophils were higher in number in the males than in the females of the control group and of the dietary groups B, C and D, while higher value was recorded in the female rats of dietary group E (Table 3). Neutrophils were more in the males of the control group and dietary groups C and E (Table 4). Female rats recorded higher lymphocyte values in the control, dietary groups B, C and D, while in dietary group E, the male rats recorded a higher value. Eosinophils were not detected in the control group but the value was higher in male rats of all the dietary groups (Table 3). Table 4 shows that the female rats had higher values of eosinophils in the control and the dietary group C, while the male rats had higher values in dietary groups B, D and E. The increase observed in the number of eosinophils and lymphocytes is indication of allergic reaction in the body. which treated metals as foreign bodies and the immune system was alerted to take care of the allergic situation.

The lymphocyte/neutrophil ratio in heavy metal-free feed experiment of dietary group E (female) was (1.56:1), while it was (4.19:1) for male. 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 group E is 3.55:1 for females and 1.43:1 for males. Various differences observed in the haematological parameters of the rats could be attributed to the composition of the diets which are quite different from the conventional rat diets. Dietary habits play important role in the leucocyte count in humans (Ezeilo, 1974). In general, these experiments have shown that supplementing rat diets with blood meal, fish meal, soybean meal and prawn meal, which are relatively, cheap and are readily available sources of protein, would yield better returns. Based on this study, we suggest that these supplements may be added to rat diets.

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