Tropical and Subtropical Agroecosystems

EFFECT OF VARIED LEVELS OF DIETARY COPPER ON PERFORMANCE AND BLOOD CHEMISTRY OF GROWING FEMALE RABBITS

[EFECTO DEL COBRE DIETÉTICO SOBRE EL COMPORTAMIENTO Y QUÍMICA SANGUÍNEA DE CONEJAS EN CRECIMIENTO]

O. A Adu[1]*, A. Akinfemi[2] and O. A. Adebiyi[3]

*[1]*Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria, Email: adufem_bunmi@yahoo.com*
[2]Department of Animal Science, Nasarawa State University, Nigeria
[3]Department of Animal Science, University of Ibadan, Ibadan, Nigeria.
**Corresponding author*

SUMMARY

Forty weanling female rabbit of average weight of 515.4g were used to assess the effect of inclusion of different levels of copper (0, 100, 200, and 300ppm inclusion levels) on the growth rate, feed intake, blood and serum parameters of the animals. Final weight, daily weight gain, daily feed intake and feed to gain ratio increased (P<0.05) with the increase in dietary supplemented copper levels until 200ppm. Hb, PCV, RBC, WBC, neutrophils, eosinophils, monocytes and lymphocytes, blood serum parameters, cholesterol and urea were not affected (P>0.05) by dietary treatment.

Key words: Cu supplementation; rabbits; blood chemistry; performance; trace nutrient.

RESUMEN

Cuarenta conejas (515 g peso promedio) fueron empleadas para evaluar el efecto de la inclusión de cobre en la dieta (0, 100, 200 y 300 ppm) sobre el crecimiento, consumo de alimento y parámetros de suero y sangre. El peso final, ganancia diaria de peso, consumo de alimento y conversion alimenticia mejoraron (P<0.05) con la inclusión cobre hasta 200 ppm. Hematocrito, cuenta de células rojas y blancas, neutrofilos, eosinofilos, monocitos, linfocitos parametros del suero sanguíneo, colesterol y urea no fueron afectados (P>0.05) por los tratamientos.

Palabras clave: Suplementación con Cu; química sanguínea; comportamiento conejos; nutrientes traza.

INTRODUCTION

The use of copper as trace nutrient in agriculture was not recognised until 1920s, before then copper was believed to be used for medicinal purpose only (Berger, 1993). Evidence that copper is a dietary essential was however obtained in 1924 as reviewed by McDonald *et al.* (2002), when an experiment with rat showed that copper was necessary for haemoglobin formation. The majority of agricultural research on copper as growth promoter involved field demonstration with the use of small amounts of copper supplement to livestock especially pigs (Jondreville *et al.*, 2002).

The copper application reported are inclusion in drinking water, mixed with feed ingredient during the milling process (Adu and Egbunike, 2005) or administered by intravenous injection (Zhou *et al.*, 1994b). Research have reported physiological responses as good bone formation, normal blood cell formation, normal myelination of brain cell and spinal cord while its deficiency is known to cause anaemia, diarrhoea, bone disorder, impaired glucose and lipid metabolism and a low immune system (Davis and Mertz,1987).

The growth promoting ability, as well as, higher feed intake, better feed efficiency and feed conversion ratio of copper in animal have been well documented especially in the west (Cromwell *et al*., 1989; Skrivan *et al*., 2002) while little has been done on the growth performing ability of copper in the tropics.

The purpose of this study was to find out the effect of long-term feeding of dietary copper on the performance and blood characteristics of growing rabbits in the tropics.

MATERIALS AND METHODS

Experimental animal and site

Forty female weanling rabbits aged 5-6 weeks and weighing 515.4 ± 44.54g were obtained from the Rabbitry unit of the Teaching and Research Farm, University of Ibadan, Nigeria, were used in a feeding trial. Laboratory analyses were carried out at the

Animal Physiology Laboratory of the Department of Animal Science of the same University.

Pre-experimental Operations

All the 40 female weanling rabbits were individually housed in wire-meshed in-door cages for a 2-week physiological adjustment period before the commencement of the feeding trial. All the animals were fed daily at 0800 and 1600h *ad libitum* with same feed for the 2-week physiological adjustment period.

Kepromec Oral (Ivemectin®) manufactured by Kepro, B.V.of Holland with batch number 0649900 was administered through drinking water against potential ecto- and endo-parasites for two days at recommended dosage by the manufacturer.

Experimental Layout and Feeding Trial

Four experimental diets were formulated: control (diet 1) diet was formulated with non-inclusion of copper sulphate, while diets 2, 3 and 4 had 100, 200 and 300ppm inclusion of copper sulphate respectively as shown in Tables 1 and 2. The diets were used for the 20-week feeding trial divided into two physiological growing phases: weanling and grower. The diets were iso-caloric and iso-nitrogenous satisfing the nutrient requirements of the animals at the two physiological phases investigated, as recommended by NRC (1998)**.**

At the end of the 2-week physiological adjustment period, the forty rabbits were randomly allocated to each of the 4 treatment diets by weight, with each treatment having 10 rabbits. The rabbits were provided fresh, clean water and appropriate pelletised feed *ad libitum* daily at 0800 and 1600h throughout the feeding period.

Data Collection

Feed consumption for each animal was measured daily by difference between the daily feed supplied and left over, and changes in live weight of the animals were taken weekly throughout the experimental period.

Blood Collection

At the end of the experiment,blood sample was collected from the ear vein of each animal into labeled bottles, one set of which contained Ethylene diaminetetraacetic acid (EDTA), an anti-coagulant while the others were without EDTA for serum biochemistry. The blood without anti-coagulant was allowed to stand in test tube rack in the laboratory in a slanting position. The serum separated from each blood sample was then decanted after centrifugation. The sera were later analysed for serum biochemicals. The blood samples in the EDTA bottles were used for haematological analyses.

Table 1. Gross composition (%) of the weanling rabbit test diets

	Diet 1	Diet 2	Diet 3	Diet 4
Ingredients	Control	100ppm	200ppm	300ppm
Maize	51.30	51.30	51.30	51.30
Wheat offal	31.10	31.10	31.10	31.10
Groundnut cake	12.50	12.50	12.50	12.50
Fish meal	2.00	2.00	2.00	2.00
Oyster shell	0.50	0.50	0.50	0.50
Bone meal	1.75	1.75	1.75	1.75
Vitamin Premix	0.20	0.20	0.20	0.20
Salt	0.45	0.45	0.45	0.45
Methionine	0.05	0.05	0.05	0.05
Lysine	0.15	0.15	0.15	0.15
Copper (ppm)	-	100	200	300
Total	100	100	100	100
Calculated Nutrient				
Crude Protein (%)	17.20	17.20	17.20	17.20
ME (Kcal/kg DM)	3110	3110	3110	3110
Crude Fiber (%)	7.20	7.20	7.20	7.20

ME: Metabolisable Energy

Table 2. Gross composition (%) of the Growers' rabbit test diets.

Ingredients	Diet 1 Control	Diet 2 100ppm	Diet 3 200ppm	Diet 4 300ppm
Maize	32.10	32.10	32.10	32.10
Wheat offal	41.80	41.80	41.80	41.80
Groundnut cake	3.50	3.50	3.50	3.50
Palm kernel cake	20.00	20.00	20.00	20.00
Oyster shell	1.50	1.50	1.50	1.50
Bone meal	0.25	0.25	0.25	0.25
Vitamin Premix	0.20	0.20	0.20	0.20
Salt	0.45	0.45	0.45	0.45
Methionine	0.05	0.05	0.05	0.05
Lysine	0.15	0.15	0.15	0.15
Copper (ppm)	-	100	200	300
Total	100	100	100	100
Calculated Nutrient				
Crude Protein (%)	10.83	10.83	10.83	10.83
ME (Kcal/kg DM)	2906	2906	2906	2906
Crude Fiber (%)	10.83	10.83	10.83	10.83

ME: Metabolisable Energy

Haematology analyses

Packed Cell Volume (PCV) was determined by spinning about 75µl of each blood sample in heparinised capillary tube in a haematocrit centrifuge for about 5 minutes and read on haematocrit reader as described by Benson *et al*. (1989) while erythrocyte (RBC) and leucocyte (WBC) counts were determined using haemocytometer method as described by Lamb (1981).

The haemoglobin (Hb) concentration and the blood constants: mean cell haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined using cyanethaemoglobin method and appropriate formula respectively as described by Jain (1986), while the differential white blood counts (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were determined as described by Lamb (1981).

Serum Biochemical Analyses

The serum total proteins was determined by the Biuret method of Reinhold (1953) using a commercial kit (Randox Laboratories Ltd, U.K.), while albumin value was obtained by bromocresol green method (Doumas and Biggs, 1971). The globulin and albumin/globulin ratio were determined according to the method of Coles (1986). The serum creatinine and urea nitrogen were estimated by deproteinisation and Urease-Berthelot colorimetric methods,s respectively, using a commercial kit (Randox Laboratories Ltd., U.K.). Also the free cholesterol was determined by nonane extraction and enzymatic colorimetric methods respectively using commercial test kits (Quimica Clinica Applicada,S.A.), while the serum enzymes Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits.

STATISTICAL ANALYSIS

The design used for the experiment is Completely Randomised Design (CRD). All the data obtained were subjected to statistical analysis using analysis of variance (ANOVA) procedure of SAS (1999). The significant treatment means were compared using the New Duncan Multiple Range test option of the same software.

RESULTS

The performance and haematology of rabbits fed copper supplemented diets are shown in Tables 3 and 4. The result showed that final live weight, daily feed intake, daily weight gain and feed to gain ratio increased significantly ($p<0.05$) with the increase in dietary supplemented copper levels until 200ppm.The daily weight gain for the animal fed copper supplemented diets (diets 2, 3 and 4) were 88.7, 78.4 and 79.1%, respectively, of those fed the control diet. The haematology parameters of rabbits were insignificantly ($p>0.05$) different, likewise the blood serum (Table 5), and were all within normal range reported by Kerr, (1989) for healthy rabbits.

Table 3. Performance characteristics of female growing rabbits fed varied levels of dietary copper.

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	
	Control	100ppm	200ppm	300ppm	+SEM*
Initial live weight (g)	514.00	500.00	519.50	528.00	44.54
Final live weight (g)	1730.09[c]	1869.00[b]	2072.50[a]	2065.50[a]	51.64
Daily dry matter intake (g)	156.36[c]	166.70[b]	184.68[a]	188.19[a]	3.54
Daily weight gain (g)	8.70[c]	9.80[b]	11.10[a]	11.0[a]	2.65
Feed conversion ratio	7.8[c]	8.20[b]	8.40[a]	8.16[a]	0.59

ab: Means on same row with different superscripts differ significantly (P<0.05).
±SEM* Standard Error of Mean

Table 4. Haematological values of growing rabbits fed varied levels of dietary copper.

	Diet 1	Diet 2	Diet 3	Diet 4	
Ingredients	Control	100ppm	200ppm	300ppm	+SEM*
Packed cell volume (%)	34.31	34.61	37.41	37.64	4.49
Haemoglobin (g/dl)	11.30	11.74	12.19	12.13	1.13
Erythrocytes (10^9/l)	5.67	5.77	5.75	5.79	0.62
MCV	59.65	58.80	57.52	60.33	5.96
MCHC	31.81	31.95	32.08	32.51	1.19
MCH	19.92	20.34	20.18	20.46	1.29
Leukocytes (10^9/l)	6.68	6.96	6.62	7.22	0.93
Neutrophils (%)	38.35	39.05	38.55	39.52	3.15
Eosinophils (%)	3.30	3.49	3.55	3.62	0.68
Lymphocytes (%)	64.56	63.84	64.17	63.34	3.41
Monocytes (%)	7.58	7.46	7.40	7.56	1.01

MCV: Mean corpuscular volume,
MCHC: Mean corpuscular haemoglobin concentration,
MCH: Mean corpuscular haemoglobin.

Table 5. Serum biochemistry of growing rabbits fed varied levels of dietary copper.

	Diet 1	Diet 2	Diet 3	Diet 4	
Ingredients	Control	100ppm	200ppm	300ppm	+SEM*
Total protein(g/dl)	6.74	6.56	6.98	6.84	0.58
Albumin (g/dl)	3.55	3.30	3.80	3.70	0.39
Globulin (g/dl)	3.29	3.25	3.26	3.14	0.39
Albumin/Globulin	1.08	1.02	1.17	1.18	0.31
Cholesterol (mg/dl)	34.31	35.20	38.30	37.02	5.11
Creatinine (mg/dl)	1.20	1.23	1.26	1.25	0.32
Urea (mg/dl)	18.16	17.80	18.14	18.16	4.77
ALT (IU/l)	51.23	51.68	52.50	50.73	1.86
AST (IU/l)	63.00	62.65	62.20	62.85	0.27

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

DISCUSSION

Braude (1948) and Barber *et al*. (1965) reported that copper added to diets at substantially higher levels than the animal requirements have shown growth promoting effect in pigs. A considerable number of experiments have been performed on animals of different body weights, ages and levels with various results. Braude (1967), in a review of the effect of an extra dietary supply of 250mg/kg in feed on an air dried basis from 25kg weight up to slaughter recorded improvement in daily body weight gain to be 8.1% on the average, a value lower than the 11.3 - 21.6% observed in this study for rabbits on copper supplemented diets.

Cromwell (1999) also reported a similar improved growth rate of 11.9%, feed intake by 8.3% and feed conversion by 4.5% in weanling pigs fed high copper diet. This author showed that the most effective level of copper in the diet was in the range of 200 to 250ppm and this agreed with the result of this study as dietary copper of 200-250ppm had a better growth rate, feed intake and feed conversion.

The difference in the feed efficiency for animals on diets 2, 3 and 4 (4.8, 7.1 and 4.41%) as compared to those on the control diet in this study was however lower than 16.8% reported by Coffrey *et al.* (1994) and 14.3% reported by Apgar and Kornegay (1996) but similar to 5.4% reported by Braude (1967) for extra dietary supply of 250mg/kg Cu in feed. Smith and Henman (2000) reported that improvement on growth rate was observed in pre-pubertal (grower) pigs fed dietary copper by increasing feed intake. This is, however, in agreement with the finding in this study, which showed that feed intake was significantly influenced by increased dietary copper.

Evidence that copper produces a growth promoting effects through the microbial gut flora have been reported (Shuron *et al*. 1990). The mechanisms involved remain not well understood. Shuron *et al*. (1990) also observed a positive effect of high concentration (283ppm) of copper in the diet on the daily growth rate and feed conversion rate in pigs and a negative effect in germ-free pigs. A similar growth-promoting effect was also obtained when copper (250ppm) in histidine solution, were administered by intravenous injection and thus by pass the gastro-intestinal tract (Zhou *et al*., 1994b).

All the haematological and blood serum parameters obtained across the treatments were within the normal range for rabbits (Mitruka and Rawnsley, 1977; Kerr, 1989) and this indicated that dietary copper does not affect the status of these blood parameters. This result agreed with the reports of Adu (2004) and Ahmed *et al.* (1997) who reported that diets supplemented with copper had no effects on blood values when fed to rabbits.

The numerically increased RBC and Hb values though not significant could be as a result of the subsequent production of more copper transporting protein ceruloplasmin, which is required for normal RBC formation by allowing more iron absorption from the small intestine and release of iron in the tissue into the blood plasma as observed by Cromwell. (1989). Ceruloplasmin had been reported (Osaki *et al*.,1966, 1971; Freiden and Hsieh, 1976) to play a critical role in the haematopoietic process, by facilitating the mobilization of iron from the reticuloendothelial cells of the liver and spleen to the bone marrow cells and by catalyzing the oxidation of ferrous iron ions during the formation of ferritransferrin. Bassuny (1991) reported similar result in a rabbit experiment conducted where the values of the RBC and Hb were higher in animals fed dietary copper compared to the control.

The non-significant effect of supplemented dietary copper on the total protein and serum albumin among the treatments in this study could probably be due to the isonitrogenous nature of the diets. These results are congruent to the previous reports that albumin levels tend to remain constant throughout life after reaching a maximum at 3 weeks of age (Miller *et al*, 1961). No significant effect was also observed in the serum globulin content and these observations concurred with the findings of Kerr *et al*. (1982) who reported no significant influence on serum globulin in sheep with high level of copper poisoning during grazing.

Non-significant differences were noticed in the serum urea values and this is due to the isonitrogenous nature of the experimental diets. The findings in this work was in line with the work of Bolourch *et al*. (1985) who reported that the nature of dietary protein influenced blood urea levels as the varying levels of urea observed among treatments could be due to the nature of the amino acids in the protein thereby varying the quality of proteins in the experimental diets.

The cholesterol values obtained in this study which was not significant among the treatments, were within the normal range of 10-80mg/dl reported by Mitruka and Rawnsley (1977) and Kaneko (1989) for healthy rabbits. This might probably be due to the fact that 6-10 % dietary crude fiber limit in rabbit diets was not exceeded. Braude (1967) had reported that crude fibre influences cholesterol levels in animals.

The normal values recorded for AST and ALT were indicative of normal functioning of the livers of the experimental animals as higher levels is as a result of extensive tissue destruction thereby liberating these enzymes into the serum leading to a diseased states as manifested in the sera of patients with acute hepatic disease (Adu, 2004).

CONCLUSION

The results of this study showed that dietary copper generally improved feed intake, and weight gains in growing rabbits. The haematological and serum biochemical parameters showed no detrimental effect. The study also showed that dietary copper at a concentration of about 300ppm for a long-term (5 month period) was not a potential health risk.

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