Effect of dietary copper supplementation on serum biochemical and histopathology of pubertal boars

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Abstract

The effects of dietary copper on some biochemistry and histopathology of organs of pubertal pigs were assessed using 32 large white male weanling pigs of 8-9 weeks of age in a 6-month feeding trial. The animals were randomly assigned to four diets containing 0, 100, 200 and 300ppm Cu/kg as the control diet (diet 1), diets 2, 3 and 4 respectively. The feeding trial was divided into 3 growth phases (weaning, pre-pubertal and pubertal) corresponding to 8, 18 and 21 weeks of life respectively. At the end of the feeding trial, all the animals were sacrificed by stunning, bled, decapitated followed by blood collection and careful evisceration to obtain the kidneys, liver, spleen and testes and sampler of the small intestine. Selected organs and tissues collected from sacrificed animals were processed for histology. Results showed that dietary copper did not significantly ($P > 0.05$) influence the blood serum biochemical composition and the histomorphology of the organs and tissues examined. The boars exposed to the diet containing the highest copper supplementation (diet 4) did not have severe splenic atrophy and/or lymphoid depletion, liver necrosis and/or lesions, intestinal mucosal erosion and testicular necrosis and/or sertoli cells degeneration as compared to those fed the control. There was no intestinal mucosal erosion with increased dietary copper in this study because the maximum level of copper that was used in this study was not above 300ppm; hence all the tissues studied did not show degeneration of any form.

Key Words: Serum biochemical, histopathology, pubertal boars, dietary copper

Introduction

Copper is a unique element in that when it is included in the diets of pigs at a relatively high level (much higher than the nutritional requirement), it stimulates growth and food conversion as much as antibiotics do. This growth stimulating effect is more evident in young pigs (Cromwell, 1999). Getting nursery pigs off to a good start can mean substantial

economic benefits to producers. Boosting growth rate of pigs will definitely reduce the time needed to get pigs to market weight. Research findings show that each additional weight gained in the nursery can cut total feeding time by several days and subsequently reduce input and operating costs (Cromwell, 1999).

Copper is an essential trace mineral, which is required by animals to support the biological

processes of life and is found throughout the body (Cromwell, 1999). Although copper has many diverse functions in the body, the major role of copper is as a constituent of a number of important metalloenzymes (Cromwell, 1999).

Drumke (1948) and Barber *et al.* (1965) reported that copper added at a higher level than normal requirement has a growth promoting effect and a lot of work has been done primarily using pigs but with little on its effect on serum and histology of the animals.

This work was therefore undertaken to study the effect of varied high dietary copper levels on serum chemistry and histological features of selected organs.

Materials and Methods

Experimental Site

The feeding trial was carried out at the Teaching and Research Farm, University of Ibadan, Ibadan and further laboratory analyses carried out at the Animal Physiology Laboratory of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Experimental Animals

Thirty-two cross-bred (Large White X Landrace) male weanling pigs aged 8-9 weeks averaging 7.49 ± 0.56 kg were sourced from the Department of Livestock Services of the Ministry of Agriculture and Natural Resources, Ondo, Ondo State, Nigeria.

Experimental Diets

Four experimental diets were formulated: control (diet 1) had non-inclusion of copper sulphate,

while diets 2, 3 and 4 had 100ppm, 200ppm and 300ppm inclusion of copper sulphate respectively as shown in Tables 1-3. The diets were used in the 6-month feeding trial. The diets were isocaloric and isonitrogenous and satisfied the nutrient requirements of the animals at the various physiological phases as recommended by NRC (1998).

Pre-experimental Operations

All the animals were individually housed in pens for a 2-week physiological adjustment period before the commencement of the feeding trial.

Kepronec Oral (Ivermectin[®]) was administered through drinking water against potential ecto- and endo-parasites for two days at recommended dosage by the manufacturer. All the animals were fed daily at 0800 and 1600h *ad libitum* with same feed for the 2-week physiological adjustment period so as to get them used to the feeding regime.

Experimental Layout and Feeding Trial

At the end of the 2-week adjustment period, the 32 animals were randomly allocated to each of the 4 treatment diets by weight, such that there was little or no variation among the mean weights of the treatments. Each treatment comprised 8 animals. The feeding trial lasted for a period of 6 months. The animals were provided clean water and the appropriate feeds *ad libitum* daily at 0800 and 1600h throughout the feeding period. The diets were fed for 6, 10 and 8 weeks for each of the phases i.e. weanling, prepubertal and pubertal phases respectively. The animals were placed under good hygienic conditions throughout the 24-week feeding period.

Table 1: *Gross composition (%) of the growing pig test diets*

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4
	Control	100ppm Cu	200ppm Cu	300ppm Cu
Maize	58.90	58.90	58.90	58.90
Groundnut cake	27.50	27.50	27.50	27.50
Wheat bran	10.00	10.00	10.00	10.00
Lysine	0.15	0.15	0.15	0.15
Methionine	0.05	0.05	0.05	0.05
Bone meal	2.00	2.00	2.00	2.00
Oyster shell	0.75	0.75	0.75	0.75
Vitamin Premix	0.20	0.20	0.20	0.20
Salt	0.45	0.45	0.45	0.45
Copper Sulphate	0.00	0.01	0.02	0.03
Total	100.00	100.00	100.00	100.00
<i>Calculated Analysis</i>				
Crude Fibre (%)	6.35	6.35	6.35	6.35
Crude Protein (%)	20.20	20.20	20.20	20.20
DE ¹ (Kcal/kg)	3350	3360	3360	3360
Copper (ppm)	-	100	200	300
Digestible Energy				

Table 2: *Gross composition (%) of the pre-weanling pig test diets*

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
	Control	100ppm Cu	200ppm Cu	300ppm Cu
Maize	51.50	51.50	51.50	51.50
Groundnut cake	12.50	12.50	12.50	12.50
Wheat offal	31.10	31.10	31.10	31.10
Fish meal	2.00	2.00	2.00	2.00
Bone meal	1.75	1.75	1.75	1.75
Oyster shell	0.50	0.50	0.50	0.50
Vitamin Premix	0.20	0.20	0.20	0.20
Salt	0.45	0.45	0.45	0.45
Copper Sulphate	0.00	0.01	0.02	0.03
Total	100.00	100.00	100.00	100.00
<i>Calculated Analysis</i>				
Crude Fibre (%)	7.20	7.20	7.20	7.20
Crude protein (%)	17.20	17.20	17.20	17.20
DE ¹ (Kcal/kg)	3110	3110	3110	3110
Copper (ppm)	-	100	200	300
Digestible Energy				

Table 3. *Diet composition, % of the pellets in test diet*

	Diet 1	Diet 2	Diet 3	Diet 4
Ingredient	Control	100 ppm Cu	200 ppm Cu	300 ppm Cu
Molass	32.30	32.30	32.30	32.30
Groundnut cake	3.50	3.50	3.50	3.50
Wheat bran	41.80	41.80	41.80	41.80
Palm kernel cake	20.00	20.00	20.00	20.00
Bone meal	0.25	0.25	0.25	0.25
Oyster shell	1.50	1.50	1.50	1.50
Vitamin Premix	0.20	0.20	0.20	0.20
Salt	0.45	0.45	0.45	0.45
Copper sulphate	0.00	0.01	0.02	0.05
Total	100.00	100.00	100.00	100.00
<i>Calculated Analysis</i>				
Gross Fibre (%)	10.80	10.80	10.80	10.80
Gross Protein (%)	15.00	15.20	15.20	15.20
DE ¹ (Kcal/Kg)	2900.00	2900.00	2900.00	2900.00
Copper (ppm)	-	100	200	300

¹ Digestible Energy.

Data Collection

At the end of the feeding trial, all the boars were sacrificed by stunning and decapitation and carefully eviscerated to collect some organs (the kidneys, liver, spleen and testes) and samples of small intestine. Selected organs and tissues collected from sacrificed animals were processed for histology as already reported by Ughurlike and Steinbach, 1972.

Chemical Analyses

The serum total protein was determined by the Biuret method of Reinhold (1953) using a commercial kit (Randox Laboratories Ltd, U.K.), while albumin values were obtained by the bromocresol green method (Doumas and Biggs, 1971). Globulin and albumin-globulin ratio were

determined according to the method of Coles (1936). Serum creatinine and urea nitrogen were estimated by deproteinisation and Urease-Berthelot colorimetric methods 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 Aplicada, 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

Completely Randomised Design (CRD) was used for the experiment. All the data obtained were subjected to statistical analysis using the analysis of variance (ANOVA) procedure of SAS (1999).

The significant treatment means were compared using the Duncan option of the same software.

Results and Discussion

The serum biochemistry of pubertal boars fed dietary copper is as shown in Table 4. All the parameters studied were not influenced by the dietary copper ($P>0.05$).

Details of the result of the histopathology of organs of pubertal boars fed dietary copper as shown in Table 5 showed that there was no kidney necrosis/dilation, intestinal mucosal ulceration, splenic atrophy as well as testicular necrosis/seminal cells degeneration in all the experimental animals fed the control diet and dietary copper up to 200mg in their diets.

Serum total protein concentrations showed no significant differences ($P>0.05$) among treatments. Values observed in this study were found to be within the normal range of 5.2-8.1g/dl reported by Friendship *et al.* (1984) as the normal total serum protein values for domestic swine.

The serum albumin also revealed no significant difference among treatments ($P>0.05$). This could probably be due to the isonitrogenous nature of the diets. These results are in agreement 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 ($P>0.05$) was noticed in the serum globulin content and this observation concurred with the findings of Kerr and McCartin (1991) who reported no significant influence on serum globulin in sheep with high level of copper poisoning during grazing.

No significant differences were noticed in the serum urea values for the pubertal boars and this could also be due to the isonitrogenous nature of the experimental diets. The findings in this work are in agreement with the work of Bolouch *et al.* (1985) who reported that the nature of dietary protein influenced blood urea levels of normal subjects as the varying levels of urea observed among treatments could be due to the nature of the amino acids in the protein thereby

Table 4: Serum biochemistry of pubertal boars fed varied levels of dietary copper

Parameters	Diet 1 Control	Diet 2 100ppm	Diet 3 200ppm	Diet 4 300ppm	SEM
Total protein (g/dl)	6.67	6.87	6.69	6.67	0.31
Albumin (g/dl)	2.92	3.06	2.97	2.98	0.04
Globulin (g/dl)	3.86	3.81	3.72	3.69	0.02
Albumin/Globulin	0.75	0.80	0.79	0.80	0.01
Cholesterol (mg/dl)	110.63	108.25	106.49	109.15	0.07
Creatinine (mg/dl)	1.95	1.87	1.91	1.89	0.03
Urea (mg/dl)	9.80	10.15	9.66	8.94	2.67
ALT ¹	20.23	20.17	20.40	21.21	0.29
AST ²	40.83	41.22	42.00	40.83	0.30

ALT¹: Alanine aminotransferase, AST²: Aspartate aminotransferase

Table 5: *Histopathological changes in goats fed with different levels of dietary copper*

Parameters	Diet 1 Control	Diet 2 100ppm	Diet 3 200ppm	Diet 4 300ppm
Spleen atrophy (lymphoid depletion)	0	0	0	12.5
Liver necrosis (lesion)	0	0	0	12.5
Kidney lesion (necrosis)	0	0	0	10
Intestinal mucosal erosion	12.5	0	0	10
Testicular necrosis, Sertoli cells degeneration	0	0	12.5	0

varying the quality of proteins in the experimental diets.

The cholesterol values obtained in this study though not significantly different among the treatments ($P>0.05$) were within the normal range of 76-174 mg/dl reported by Mitruka and Rawnsley (1977). This might probably be due to the fact that the 10-15% dietary crude fibre limit in swine diets was not exceeded as Brude (1967) reported that crude fibre influences cholesterol levels.

The findings of this study showed that there were no significant variations ($P>0.05$) in the values obtained for AST and ALT. The values obtained for AST were in consonance with the range of 37-46U reported by Gouercie and Gouercie (1996) and 15-45U for ALT by Friendship *et al.* (1984) for normal pigs. The normal values returned for AST and ALT were indicative of normal functioning of the livers of the experimental animals as extensive tissue destruction, these enzymes are liberated into the serum thereby leading to higher levels in diseased states as manifested in the sera of patients with acute hepatic disease (Cornelius, 1970).

The histopathology results showed that all the parameters studied did not have any

degeneration of any form and this could be probably because the level of dietary copper did not exceed 300ppm. Cromwell *et al.* (1998) reported that it is only higher levels of inclusion of copper from 500ppm and above and that it is animals that are sensitive to copper for example sheep that have been observed to have lesions, intestinal erosion, splenic atrophy depending on the stage it is fed. The author fed pigs at 0, 250, 350 and 500ppm of dietary copper to arrive at this conclusion and also reported depressed growth and in some instances toxicity and death at this higher level. The toxicity probably resulted from excessive copper accumulation in the liver, which causes breakdown in liver function and sudden death (Zorvas *et al.* 1990).

The report of Cromwell *et al.* (1998) agreed with the work of Boyden *et al.* (1998) who fed 21 day-old albino rats *ad libitum* for 4 weeks with diets containing 0, 500, 1000, 2000 and 4000 ppm copper as copper sulphate. The authors reported that at dietary copper levels of 500 ppm and above, decreased daily feed intake as dietary copper increased. All the rats in the 4000 ppm treatment died within the first week while one of eight animals in the 2000ppm treatment group died during the fourth week. Histological

examination revealed degeneration of liver parenchymal cells with marked fibrosis, tubular necrosis of the kidneys, nerve cell degeneration, and swelling of the brain stem. Narasaki (1989) also reported granular copper deposits in liver parenchymal cells, in glia cells of the central nervous system and in the degenerated tubular epithelial cells of the kidneys when weanling male Wistar rats received 1.5mg Cu/kg daily injection in a 160 days study period.

Copper sulphate at 530 and 1600 ppm copper, respectively and copper gluconate at 1600ppm were fed in the diet of rats for up to 44 weeks. A control group was also maintained. Each treatment group consisted of approximately 25 male and 25 female rats.

Significant growth retardation, which was discernible at the twenty-sixth week, occurred in the high level copper sulphate and the copper gluconate groups. Mortality, which was elevated in the high level copper sulphate treatment group, was up to 90% between the fourth and eighth month in the copper gluconate group. Animals receiving copper gluconate lower than 500ppm had hypertrophied uteri, ovaries and seminal vesicles while both high level copper sulphate- and copper gluconate-fed animals showed enlarged, distended and hypertrophied stomachs, occasional ulcers, bloody mucus in their intestinal tract, and bronzed kidneys and livers. Histopathological examination of these animals showed abnormal hepatic and renal changes, as well as varying degrees of testicular damage. The following wet weight copper levels were reported in the livers from male and female rats respectively from the various treatment groups. After 40 weeks, 1.6 and 17.8ppm (Controls),

124.7 and 323.6ppm (530ppm CuSO_4), 378.8 and 457.3ppm (1600ppm CuSO_4), and after 30 weeks 751.0 and 566.0ppm (1600ppm copper gluconate). A marked depression in tissue storage of iron in the high-level copper sulphate and copper gluconate animals was also noted. It was concluded that 1600 ppm copper either as copper sulphate or copper gluconate was toxic while 530 ppm copper as the sulphate caused only variable effects on testicular degeneration and tissue storage of copper (Harrison *et al.*, 1954). These findings were in agreement with what was observed in the present study because the maximum level of copper that was used in this study was not above 500ppm; hence all the tissues studied did not show degeneration of any form.

Conclusion

It can be concluded that dietary copper between 100-300ppm did not prompt abnormal hepatic changes, testicular degeneration, intestinal erosion and lesion, splenic atrophy and growth rate was not retarded.

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