

Comparative effect of organic and Inorganic Copper Supplemented Diets on Performance and Blood Characteristics of Growing Rabbits

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Abstract

The effects of organic and inorganic copper sources on the performance and blood characteristics of growing rabbits over a 10 week period were investigated. Six dietary treatments were formulated such that diets 1, 2 and 3 contained 10, 50, and 100ppm of organic copper (Copper proteinate (Cu Pro)) while diets 4, 5 and 6 contained 10, 50 and 100ppm of inorganic copper (copper sulphate ($CuSO_4$)). The final weight and daily weight gain were significantly ($P<0.05$) higher in rabbits fed organic Cu compared to those fed $CuSO_4$. Organic Cu diets resulted in significant ($P<0.05$) reduction in blood cholesterol than $CuSO_4$ diets. Organic Cu is more effective in promoting growth and blood cholesterol reduction of growing rabbits than $CuSO_4$. There was more accumulation of Cu in the liver and heart of rabbits fed Organic Cu than those fed $CuSO_4$. The liver and heart Cu increased as the Cu level increased. More Cu was excreted in rabbits fed $CuSO_4$ than those fed Organic Cu. This study showed that copper proteinate is more effective in promoting growth and reduction of cholesterol of rabbits. The study confirmed a relative increase in bioavailability of organic copper compared to inorganic sources.

Keywords: Rabbits, copper proteinate, copper sulphate,

Introduction

The rising demand for animal products occasioned by increasing population has led to increasing cost of the product, thus making it out of reach of average masses. Attention has recently been diverted to rabbit production due to their high production capability and short production cycle, as one of the fastest ways of meeting the increasing demand for the products. Rabbits are animals that are less expensive, easy to manage, quiet and restful (Katie *et. al.*, 1986). They can be raised on high fiber feeds and materials not utilized by man. Since they do not compete with man for grains, they have the advantage over swine and poultry. Optimizing rabbit production to meet the nutritional need of the masses requires among other inputs a balanced nutrition. In formulating diets for rabbits, much attention has been given to major nutrients such as protein and energy as well as some macro minerals such as calcium and phosphorus. Less attention is often given to trace minerals, their role is often underestimated and their presence in adequate quantity is usually taken for granted. Copper is one of the essential trace minerals in animal nutrition. It is required for the proper functioning of the central nervous system, immune and cardiovascular systems and in pigmentation of the skin (Close, 1998). Cu is an essential component of several enzyme systems, especially metalloenzymes which are important for cellular respiration. Cu supplementation in animal diets has been traditionally achieved through the use of inorganic salts such as $CuSO_4$. These salts are broken down in the digestive tract to form free ions which are absorbed. However, free ions are very reactive and can form complexes with other dietary molecules that are difficult to absorb (Close, 1998). There has been increasing interest in the use of organic or chelated copper. This is the form in which it occurs in nature. Recent studies suggest that organic sources of Cu may be more reactive in promoting growth than $CuSO_4$. Work with rats showed organic Cu are better utilized than $CuSO_4$ (Du *et al*, 1996). This study was carried out to investigate the utilization of organic and inorganic Cu sources and their effects on the performance and some blood characteristics of growing rabbits.

Materials and Methods

Experimental Site

The research work was carried out at the rabbit unit of the Teaching and Research Farms, University of Agriculture, Abeokuta, Nigeria (7^0 10^1 N and 3^0 2^1 E).

Copper Sources

The copper sources used are copper proteinate and copper sulphate. The copper proteinate was obtained from Alltech® Inc. USA. It contained 10% copper. Feed grade copper sulphate used was obtained from Lucaris Limited, Lagos, Nigeria. It contain 25% copper. Other feed ingredients were purchased from a reputable commercial feed mill in Abeokuta.

Management of Experimental Rabbits

Forty-eight growing rabbits of mixed breeds and sexes purchased from a reputable rabbit farm in Abeokuta were used for this study. The rabbits were between 7-8 weeks of age and their weight ranged from 670-820g. On arrival at the rabbit unit, they were given antibiotics, mineral vitamin mix and dewormer. The rabbits were housed in wooden cages with metal mesh floors that allows for separate collection of urine and faeces. The cages were thoroughly disinfected before stocking. Each cage was provided with a concrete feeder and drinker. The house was designed to ensure cross ventilation and exclude rodents and other pests.

Experimental Design

Forty-eight rabbits were randomly allotted to 6 treatments of 8 rabbits each. Each treatment group was further divided into 4 replicates of 2 rabbits each. The treatment comprised 6 experimental diets in a 2x3 factorial design consisting 2 Cu sources (Cu proteinate (Organic Cu) and Cu sulphate ($CuSO_4$)) each at 3 levels 10, 50 and 100ppm. Diets 1, 2 and 3 contained 10, 50 and 100ppm Cu Proteinate while diets 4, 5 and 6 contained 10, 50 and 100ppm $CuSO_4$ respectively. The composition of experimental diets in g/kg is shown in Table 1. The premix used was Copper free.

The study lasted for 10 weeks during which data on growth indices such as body weight, weight gain, feed intake and feed conversion ratio were collected weekly

The background Cu content of the diets was determined before supplementation.

Chemical Analysis

The proximate composition of the basal diet was determined by the method described by AOAC (1995) and background copper content of the basal diet, test diets and faeces were determined by wet-ashing procedure (James,1996). The moisture content of the samples was oven-dried at 65°C for 36 hours.

Biochemical, Haematological and Cholesterol Analysis

At the 10th week of the study, blood samples were collected through the neck sliting. 2.5ml of blood was collected from a rabbit per replicate into tube containing Ethylene diamine tetra acetate (EDTA) and another 2.5ml was collected into a

Table 1: Composition of basal experimental diets (gkg^{-1})

Ingredients	**% Composition**
Maize	475.00
Soybean meal	60.00
Groundnut cake	80.00
Wheat offal	350.00
Bone meal	20.00
Oyster shell	10.00
Salt	2.50
Premix	2.50
Total	**1000.00**
Determined Analysis	
Nutrients	
Energy (ME $MJkg^{-1}$)	11.46
Protein	151.50
Fat	11.50
Ash	153.50
Fiber	147.80
Calcium	10.90
Phosphorus	3.00
Background copper in the diet (ppm)	25.91

Vitamins /mineral premix (Godomix) based on 2.5kg per ton.

Growers premix composition

Vit. A: 3,200000IU, vit.D: 640000 IU, vit E: 2000mg, vit k_3: 800mg, vitB_1: 2000mg, vit B_2: 6000mg, vit. B_6: 5000mg, vit B_{12}: 25mg, Niacin. 6000mg, Panthotenic Acid 20000mg, Folic Acid. 1000mg, Biotin. 8mg, Manganese: 30000mg, Iron. 20000mg, Zinc: 20000mg, Copper Nill, cobalt: 80mg, Iodine: 480mg, Selenium:40mg, Choline: 800000mg BTH 25,000mg Anticaking agent: 6,000mg hypodermic syringe. The blood in EDTA bottles were used to determine haematological parameters (Packed cell volume(PCV),Red blood cell(RBC),White blood cell (WBC) and Haemoglobin(Hb) while those in hypodermic syringe were used to determine serum parameters (Serum total protein, Albumin, Globulin, Urea and Creatinine). The PCV was determined by microhaematocrit method (Baker and Silverton, 1985) while Hb and RBC were determined using colorimetry cyanomethaemoglobin and improved Neubauer haemocytometer methods respectively (Jain, 1986). The Serum total protein, Albumin and Globulin were analysed colorimetricaly using diagonistic reagent kit (Varley *et al*, 1980) .Plasma was separated from the blood samples in EDTA bottles with a micro pipette into a test tube for triglyceride and cholesterol analysis. The cholesterol and triglyceride assay of the blood plasma were done by enzymatic- colorimetric methods (according to manufacturer's manual) using Randox^R diagonistic cholesterol kit (BIOLAB with code 80106.2 x 100ml cholesterol CHOD-PAP^R) and Randox^R diagonistic triglyceride reagent procedure (GPO-PAP Method Randox Laboratory Ltd, UK) respectively.

Copper bioavailability analysis: - Visceral organs (liver, heart and kidney). muscles and bones of rabbits were excised and stored at -2°C. The samples were dried at 100°C for 36 hours and digested using a modified wet-ashing procedure. Ash was reconstituted in 4m/s of 1*N* HCL solution and analysed for copper via atomic absorption spectrophotometry. (James, 1996).

Statistical Analysis

Data obtained were subjected to 2x3 factorial Analysis of Variance (Steel and Torrie, 1980) using MINITAB statistical software (MINITAB, 2000). Significant means were separated using Duncan's multiple range test (Duncan, 1955.).

Results and Discussion

Table 2 shows the main effect of Cu sources on the performance of growing rabbits. The final weight (FW), daily weight gain (DWG) and the feed conversion ratio (FCR) were significantly ($P<0.05$) higher in rabbits fed organic Cu than those fed $CuSO_4$. Zhou *et al*, (1994) reported a significantly higher growth rate for pigs fed organic Cu than $CuSO_4$. Close, (1998) reported 10.8% improvement in growth rate of pigs fed Cu proteinate compared to those fed $CuSO_4$. The results of this study attest to the fact that organic Cu has a higher growth promoting potential than $CuSO_4$ Significantly higher ($P<0.05$) feed conversion ratio (FCR) value was reported for rabbits fed $CuSO_4$ supplemented diets. The lower FCR value of rabbits fed organic Cu based diets is an indication that the diets was better utilized by the rabbits. The interaction of Cu sources and Cu levels (Table 3) produced no significant effect ($P>0.05$) on growth rate of the growing rabbits

Table 4 shows the main effect of Cu sources and Cu levels on blood parameters of growing rabbits. The Hb value was significantly higher in rabbits fed Organic Cu diets. Cu and Fe are known to play vital roles in the synthesis of heamoglobin and for the synthesis of enzyme needed for normal metabolism (Close, 1998).

Table 2 : Main effect of copper sources and copper levels on the performance characteristics of growing rabbits (g).

Parameters	Copper Sources			Copper levels			
	Organic Cu	CuSO4	SEM	10ppm	50ppm	100ppm	SEM
Initial weight.	747.92	720.00	11.13	748.45	703.7	718.3	12.31
Final weight.	1769.92[a]	1615.30[b]	13.67	1631.30	1559.40	1574.00	62.42
Daily weight gain	14.60[a]	12.79[b]	0.12	12.61	12.34	12.23	0.57
Daily feed intake	61.42	56.16	1.24	57.15	54.40	59.82	1.62
FCR	4.21[a]	4.39[b]	0.09	4.53	4.41	4.89	0.2

[ab]means in the same row having the different superscripts are significant ($P<0.05$)
SEM: Standard Error of Means.

Table:3 Interactive effect of copper sources and copper levels on the performance characteristic of growing rabbits

Parameters	**Organic Cu**			$CuSO_4$			**SEM**
	10ppm	50ppm	100ppm	10ppm	50ppm	100ppm	
Initial weight.	750.00	750.00	743.77	746.90	657.43	692.73	36.40
Final weight.	1850.00	1564.60	1668.77	1812.50	1554.17	1479.19	110.40
Daily weight gain	15.71	11.88	13.21	15.21	12.81	11.24	1.27
Daily feed intake	60.81	57.50	65.97	63.50	51.30	53.67	2.88
FCR	3.90	4.87	4.99	4.18	4.08	5.10	0.41

SEM: Standard Error of Means

Table 4: Main effect of copper sources and copper levels on the blood parameters of growing rabbits

Parameters	**Copper sources**			**Copper levels**			
	Organic Cu	$CuSO_4$	SEM	10ppm	50ppm	100ppm	SEM
Packed cell volume (%)	36.33	34.33	0.67	33.50	33.25	39.25	1.39
Haemoglobin (g/dl)	12.38[a]	11.65[b]	0.07	11.40	11.35	13.30	0.45
White blood cell ($10/mm^3$)	12.87	13.40	0.14	12.20	13.98	13.35	0.37
Red blood cell ($10/mm^3$)	12.67	11.99	0.16	11.68	11.77	13.57	0.44
Serum total protein (g/l)	57.17	60.73	0.84	56.88[b]	58.28[b]	61.07[a]	1.07
Serum albumin (g/l)	35.20	38.52	0.78	36.80	36.98	36.80	0.04
Serum urea (g/l)	5.42[a]	4.45[b]	0.23	5.18[a]	4.80[b]	4.83[b]	0.09
Serum creatinine (mg/dl)	1.18	1.20	0.03	1.15b	1.28[a]	1.15[b]	0.03
Cholesterol (mg/dl)	128.67[b]	154.85[a]	6.17	164.30[a]	146.77[b]	114.28[c]	10.36

[ab]means in the same row having the different superscripts are significant ($P<0.05$)
SEM: Standard Error of Means

The relatively higher Hb in the blood of rabbits fed Organic Cu could be attributed to the availability of Organic Cu for haemoglobin synthesis. Rabbits fed $CuSO_4$ supplemented diets had significantly higher serum urea and cholesterol values than those fed Organic Cu supplemented diets. Serum uric acid has been identified as one of the indicators of protein quality. Organic Cu may have direct or indirect influence on protein utilization. The lower value of cholesterol recorded by rabbits fed Organic Cu showed that proteinate form of Cu was more effective in reducing cholesterol level than sulphate when fed to rabbits. This observation agreed with Pesti and Bakalli, (1988) who reported that sulphate form of Cu resulted in higher values of cholesterol than other forms. Paik *et al,* (1999) also reported that dietary intake of Cu chelate reduced total plasma cholesterol. This observation may be due to the fact that Cu regulates cholesterol biosynthesis by reducing hepatic glutathione concentration (Kim *et al,* 1992). The serum cholesterol decreased with increased concentration of Cu. This suggests that feeding high Cu concentration significantly reduces serum cholesterol in broilers.

Copper Accumulation in Visceral Organs

Table 5 shows the main effect of Copper sources on copper accumulation in visceral organs, faeces and blood of growing rabbits. The result shows that copper in the liver, heart and faeces of the rabbits were significantly ($P<0.05$) influenced by copper sources. Rabbits fed Organic Cu had significantly higher ($P<0.05$) values of liver and heart copper compared to those fed $CuSO_4$. This suggests that Organic Cu was more readily absorbed and more distributed into these organs than $CuS0_4$. Ryan *et al,* (2002) reported that sheep supplemented with bioplex Cu level than sheep given $CuSO_4$. However, rabbits fed $CuS0_4$ based diets had significantly ($P<0.05$) higher Cu excretion. Also, copper in the kidney, lung and blood were not significantly ($P>0.05$) influenced by copper sources. The higher value of faeces Cu in rabbit fed $CuSO_4$ showed that the $CuSO_4$ was poorly absorbed hence voided. Feeding Organic Cu resulted in 45% reduction in Cu excretion compared to $CuSO_4$. This observation agreed with the report of Dirk Fremant (2003). The author reported 37.8% reduction Cu excretion of piglet fed bioplex Cu compared to those fed $CuSO_4$. Egeli *et al* (1998) observed similar report that liver copper levels were notably higher in piglets from the Cu proteinate supplemented group. In ruminants, copper proteinate has been reported to be more bioavailable than cupric sulphate in studies involving beef cattle (Hemken *et al,* 1993). This observation suggests that organic form of Cu is more bioavailable than inorganic form. Baker and Ammerman (1995) and Kincaid *et al*, (1996) in their findings considered liver Cu as a more reliable response criterion of Cu bioavailability. Also, liver was reported to be the major site for Cu metabolism (Chiou *et al,* 1998) and it is a better indicator of Cu status and relative bioavailability between sources (Lee *et al,* 1988, Xin *et al,* 1991). The blood Cu of rabbits fed Organic Cu was slightly higher ($P>0.05$) than those fed $CuSO_4$ though at no significant and blood were not significantly ($P<0.05$) influenced by copper sources.

Table 5: Main Effect of Copper sources and Copper levels on the copper accumulation in visceral organs. faeces and blood of growing rabbits (mgkg-1)

Parameters	**Copper sources**			**Copper levels**			
	Organic Cu	$CuSO_4$	SEM	10ppm	50ppm	100ppm	SEM
Liver copper	24.32[a]	18.32[b]	1.41	17.85[b]	21.11[ab]	24.99[a]	1.46
Heart copper	32.50[a]	24.39[b]	1.91	21.99[b]	33.32[a]	30.03[ab]	2.38
Kidney copper	25.90	23.01	0.61	22.23	24.86	26.27	0.84
Lung copper	21.50	21.65	0.04	23.20	19.92	21.61	0.67
Faecal copper	30.89[b]	44.84[a]	3.29	33.49	36.67	41.53	1.68
Blood copper	9.32	5.09	0.99	6.97	3.96	10.68	1.37

- [ab] means of the same row having different superscripts are significantly different (P<0.05)

SEM: Standard Error of Means

Table 6: Interactive Effect of Copper sources and Copper levels on the copper bioavailability in visceral organs, lungs, faeces and blood of growing rabbits (mgkg-1)

Parameters	**Organic Cu**			$CuSO_4$			**SEM**
	10ppm	50ppm	150ppm	10ppm	50ppm	100ppm	
Liver copper	18.38	23.48	31.11	17.33	18.73	18.89	2.14
Heart copper	25.18	34.11	38.21	18.81	32.54	21.84	4.03
Kidney copper	24.94	25.22	27.52	19.49	24.50	25.03	2.14
Lung copper	23.71	19.17	21.69	22.69	20.68	21.58	2.25
Faecal copper	30.98	29.49	32.20	35.83	47.84	50.86	3.92
Blood copper	7.18	4.83	5.96	6.76	3.10	5.41	2.55

SEM: Standard Error of Means.

The levels of copper supplementation was significantly (P<0.05) influenced by copper in the liver and heart. The rabbits fed 100ppm Cu had the highest (P<0.05) liver copper while those fed 10ppm and 50ppm had statistically similar values. Liver Cu increases as the level of Cu supplementation increases. Also, heart Cu increases as Cu level increases. Rabbits fed 50 ppm had a similar heart Cu level. Also, the rabbits fed 50ppm Cu had the highest (P<0.05) heart copper while those fed 10ppm and 100ppm had statistically similar values. The copper in kidney, lung, faeces and blood were not significantly affected by the copper levels. The trend observed was that copper in kidney and faeces slightly increased (P>0.05) as the levels of copper supplementation increased.

Table 6 shows the interactive effect of copper sources and copper levels on copper bioavailability in visceral organs, faeces and blood of growing rabbits. The result shows that the parameters measured were not significantly (P>0.05) influenced by the interaction of copper sources and copper levels.

Conclusion

The results of this study showed that copper proteinate is more effective in promoting growth and reduction of cholesterol of rabbits. The study confirmed a relative increase in bioavailability of organic copper compared to inorganic sources.

References

Baker, D.H and C.B.Ammerman. 1995. Copper Bioavailability. In: C.B Ammerman, D.H. Baker and A.J. Lewis, eds.Biavailability of nutrient: Amino acids, minerals and vitamins. Pp 127-156. Academic Press, San Diego CA.

Baker, F. J and Silverton, R. E. 1985. Introduction to medical laboratory technology. 6th edition, Butterworth & Co publishing company. Pp523-531

Chiou, P. W. S., K. L. Chen and B. Yu. 1998. Effect of dietary organic arsenicals and cupric sulphate on copper toxicity, liver accumulation and residue in eggs and excreta of laying hens. *Anim. Feed Sci. Tech.*, 73: 161-171

Close W. H. 1998: The role of trace mineral proteinates in pig nutrition. In: Biotechnology in the feed industry, proceedings of the 14th Annual Symposium (T. P. Lyons and K. A. Jacques, eds.) Notthingham University Press. Nottingham, U.K. pp 469-474.

Dirk Fremant (2003). Trace minerals proteinates in modern pig products: reducing mineral excreting without sacrificing price in mutual Biotechnology in feed and feed industry. Poceedings of the 19th Annual Symposium (T. P. Lyons and K. A. Jacques, eds.) Notthingham University Press. Nottingham, U.K. pp 172-178.

Du, Z. R. W., Hemken, J. A., Jackson, and D.S. Trammell. 1996: Utilization of copper in copper proteinate, copper lysine and cupric sulphate using the rat as an experimental model. *Journal and Animal Science,* 74: 1657-1663.

Duncan, D. B. 1955. Multiple Range and Multiple F-test *Biometrics.* 11: 1-24.

Egeli, A.K.T Franstand and D. Greeningen (1998). The effect of peroral administration of amino acid-chelated ion to pregnant sow in preventing sow and piglet anaemia. Acta *Vet. Scand.* 39: 77-87.

James, C. S. 1996. Analytical Chemistry of Foods. Blackie Academic and Professionals, Glascow.

Hemken, R. W., Clark, T. W. and Du, Z. 1993. Copper: Its role in animal nutrition. In: Biotechnology in fed industry (T. P. Lyons, Ed). Alltech technical publications, Nicholasville, Kentucky, USA, Pp 35-39.

Katie, T. and Allistair, F. 1986: The complete book of raising livestock and poultry. University of Service Limited Education Publication, Yaba, Lagos.

Kim, J. W., Chao, P. Y., and Allen, G. D. A. 1992: Inhibition of the elevated hepatic gluthione abolishes copper deficiency cholesterolemia. FASEB Journal. 6: 2467-2479.

Kincaid, R. L., R. M. Blauwiekel and J. D. Coranth. 1986. Supplementation of copper as copper sulphate or copper proteinate for growing calves fed forages containing molybdenum. *J. Dairy Sci.* 69:160-163.

Lee, D. Y., J. Schroder and P. T. Gordon. 1988. Enhancement of the bioavailability of copper in the rat by phytic acid. *J. Nutr.* 118: 712-716.

Minitab Inc. 2000. Minitab statistical software. Version 13

Paik, I.K., Seo, S. H., Um, J. S., Chang, M. B and Lee, B. H. 1999. Effect of supplementary copper-chelate on the performance and cholesterol level in plasma and breast muscle of broiler chicken. *Asian-Austr.* Vol 12 No5 :794-798.

Pesti, G. M and bakalli, R. I. 1998. Studies on the effects of feeding cupric sulphate pentahydrate to laying hens on egg cholesterol content. *Poultry Sci.* 77: 1540-545.

Steel, R. G.D and Torrie, J. H. 1980. Principle and Procedures of Statistics. McGraw-Hill Book Company, New York.

Varley, H., Gowelock, A. H. and Bells, M. 1980. Detemination of serum urea using the acetyl monoxide method. Practical biochemistry, 5th Edition. William Heinemann Medical Books Ltd. London.

Xin, Z. D. F., Waterman, R. W., R. J.. Harman and J. A. Jackson. 1991. Effects of copper sources and dietary cation-anion balance on copper availability and acid-base status in dairy calves. *J. Dairy Sci* 74: 3167.

Zhou, W., E. T. Kornegay., H. Van Laar., J. W. G. M. Swinkels., E. A. Wong and M. D. Linderman. 1994. The role of feed consumption and feed efficiency in copper-stimulated growth. *J. Anim. Sci.* 72:2385-2394.

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