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ORIGINAL RESEARCH ARTICLE

Influence of different forms of copper supplementation on West African dwarf does fed malted sorghum sprout

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ABSTRACT

This study investigated the influence of different forms of copper supplementation on West African dwarf goats to diets containing fermented malted sorghum sprout supplemented with inorganic and nano copper sources. Thirty-six pregnant does averaged 17±0.2g were used in a 90-day feeding trial to determine the response of WAD goats to different levels of dietary inorganic (IC) and nano copper (NC) supplementation of fermented malted sorghum sprout (FMSP) on growth performance, haematological and serum biochemical profile, serum minerals and oxidative stress biomarkers. Based on weight equalization each group was allotted to the 6 experimental diets containing: 0 mg/kg inorganic copper, 15 mg/kg inorganic copper, 30 mg/kg inorganic copper, 0mg/kg nano copper 15 mg/kg nano copper and 30 mg/kg nano copper in a Completely Randomized Design. Data were collected on growth performance, haematological profile, serum biochemisry, serum minerals and serum oxidative biomarkers. Goats on dietary supplementation of 30 mg/kg NC had the highest ($p<0.05$) weight gain (4.30kg), metabolic weight (2.97kgBW$^{0.75}$) and best feed conversion ratio (23.57). Animals fed FMSP supplemented with 0 mg/kg gave highest ($p<0.05$) neutrophil (36.67%), basophil (2.33%) and eosinophil (2.00%) and does on 15mg/kg copper supplementation had the highest lymphocyte (65.67%). In addition, serum cholesterol and serum urea nitrogen were influenced ($p<0.05$) by incremental levels of copper supplementation. Goats offered 30 mg/kg IC had the highest ($p<0.05$) catalase (1.35) while goats fed 0 mg/kgIC and 0 mg/kgNC had the highest ($p<0.05$) value for glutathione. Also, the dietary supplementation of inorganic and nano copper at 30mg/kgIC and 15 mg/kgNC displayed highest ($p<0.05$) serum calcium (3.10) and copper (0.09) with comparable value at 15mg/kgIC and 30 mg/kgNC. Goats fed 30mg/kg inorganic copper can be used to increase copper levels in the blood. However, in terms of daily gain, dietary supplementation of 30 mg/kg NC is adequate.

Keywords: Fermented malted sorghum sprout, inorganic copper, nano copper, ruminant nutrition.

INTRODUCTION

Nutrition is one of the most important factors limiting animal production in most part of the world, and this is more pronounced in the tropics, where ruminant animals suffer from scarcity of feed supply and pasture quality most especially during the dry season when the natural vegetation is of poor nutritive value (Ososanya *et al.*, 2013). There is a continued search for feed resources that will address these shortages. Sorghum (Sorghum *bicolor*), a staple food of the people in semi-arid zones of Africa, Asia and South America is drought tolerance (Belton and Taylor, 2004). The seed of sorghum could be malted to obtain malt with resultant residue consisting of the sorghum shoots and roots (Aletor *et al*., 1998). Malted sorghum sprout (MSP), a non-conventional feedstuff for animals is relatively new in the feed industry. Malted sorghum sprout is reported to contain a considerable number of amino acids with low level of methionine, lysine and threonine, magnesium and zinc are the most abundant mineral while potassium and copper are the least (Aning *et al*., 1998). Attempt to improve on the utilization of MSP by animals must also include enriching it with those deficient trace minerals.

Nano minerals have been produced through nanotechnology and it improve bioavailability. Aside the nano form, particles exist also in the inorganic form which could also be utilized by animals. It is therefore important to compare the utilization of both nano and inorganic particles by animals especially in trying to enrich certain feed resources. Copper deficiency in livestock feed causes insufficient weight gain, poor appetite, poor milk production, loss of hair colour, rough hair coat, muscles tremors and anemia. The variations in copper requirements at various stress condition, relationships between dietary copper source and level, related enzyme activities, growth performance, nutrient digestibility and immune function recently have attracted considerable interest among scientists (Solaiman *et al*. 2007). But the information regarding the influence of copper supplementation at different dietary concentrations from inorganic and nano copper sources on blood parameters in goats is limited. Hence, this study attempts to assess the possible effect of copper supplementation from two different forms at different levels on growth performance, haematological parameters and copper (Cu) status in West African Dwarf goats.

MATERIALS AND METHODS

The experiment was carried out at the Small Ruminant Unit of the Teaching and Research Farm of College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria and it lasted for a period of ninety days.

A total of twenty-four females (24) West African dwarf goats were obtained from local market in Abeokuta**,** Ogun state, Nigeria. The goats with average weights between 16-19 kg were randomly allotted to six dietary treatments in a completely randomized design. Copper sulphate (inorganic), nano copper and malted sorghum sprouts were used for the experiment. Copper sulphate was purchased from Rotinol International Limited Abeokuta, Ogun State, Nigeria, Nano copper was obtained from Canfuo Nanotechnology Co., Ltd, China and Malted sorghum sprout (MSP) was purchased from Life Care Ventures Limited in Sango-Otta, Ogun State, Nigeria. Malted sorghum sprout was thoroughly hand-mixed with water in the ratio 1:2 according to (Fanimo and Akinola, 2006). The mixture was then transferred into an air-tight nylon sack and fermented naturally under room temperature for four days to obtain fermented malted sorghum sprout (FMSP) and was sun-dried by spreading it on the concrete floor. Six concentrate diets were compounded with the FMSP by adding other ingredients such as maize bran, wheat offal, Cu free premix, limestone and salt and inorganic and nano copper were added to the diet at the rate of 0 mg/kg, 15 mg/kg and 30 mg/kg as indicated in Table 1.

The goats were confined in a well-ventilated individual pens that was cleaned, washed and disinfected with disinfectant prior to the arrival of the goats. On arrival, the animals were quarantined for a period of twenty-eight days during which the goats were given prophylactic treatments; consisting of intra-muscular injection of oxytetracycline LA at 1ml/25kg and Ivermectin at 1ml/25kg live weight. Guinea grass, control diets and cassava peels were fed to the animals during the adjustment period. Fresh cool clean water was also made available *ad libitum* throughout the experiment. After the adaptation period of 28 days, the animals were balanced as closely as possible for their body weights and randomly allotted into six dietary treatments.

Dry Matter Intake and Live Weight Gain:

At the beginning of the experiment, the goats were weighed and subsequently on a weekly basis prior to feeding in the morning. The initial live weight was subtracted from the final live weight to determine the weight gained by the goats. Feeds offered and remnants were weighed daily to determine the dry matter intake of the goats. Both values were used to determine Feed Conversion Ratio (FCR).

Collection of Blood Sample

Blood sample (10ml) was drawn from three randomly selected goats per treatment via the jugular vein. Approximately 5ml of blood was drawn using needle and syringe and was ejected gently into green top bottle to ensure easy mixing of the blood with the EDTA anticoagulant. The remaining blood sample (5ml) was poured into a plain sample bottle for serum analysis.

Table 1: Ingredient and composition of the experimental concentrates diet

Ingredients (%)	Inorganic copper			Nano copper		
	0 mg/kg	15 mg/kg	30mg/kg	0 mg/kg	15 mg/kg	30mg/kg
Maize bran	40.00	40.00	40.00	40.00	40.00	40.00
Wheat offal	24.25	24.25	24.25	24.25	24.25	24.25
FMSP	30.00	30.00	30.00	30.00	30.00	30.00
Premix (Cu free)	0.25	0.25	0.25	0.25	0.25	0.25
Limestone	5.00	5.00	5.00	5.00	5.00	5.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**	**100**	**100**
Determined analysis (%)						
Dry Matter	91.34	91.84	92.22	91.29	92.76	93.14
Crude Protein	13.51	14.02	13.37	13.45	13.65	14.40
Crude Fibre	2.68	3.09	3.63	2.72	4.06	5.69
Ash	6.18	7.23	11.76	6.09	12.66	14.33
Ether extract	1.91	1.44	1.12	1.86	1.01	0.88
Non-fibre carbohydrate	910.26	911.72	905.95	908.89	892.4	905.70
Neutral detergent fibre	66.46	62.50	64.17	67.57	76.22	62.00
Acid detergent fibre	56.25	49.21	54.47	55.04	50.85	47.16
Acid detergent lignin	44.22	36.39	26.45	43.11	33.22	28.41

Available copper (Cu) in 38 mg/kg of $CuSO_4$ is 15 mg/kg; Available copper (Cu) in 76 mg/kg of $CuSO_4$ is 30 mg/kg; FMSP: Fermented Malted Sorghum Sprout

The Proximate analysis of the experimental diets, FMSP and *Panicum maximum* were determined according to the official methods of analysis described by (AOAC, 2000). Data obtained were subjected to analysis of variance using 2 x 3 factorial arrangement in a completely randomized design. Significant means were separated using the LSD as contained in SAS (2000).

RESULTS AND DISCUSSION

The interactive effect of copper sources and different levels of dietary inorganic copper (IC) and nano copper (NC) supplementation of fermented malted sorghum sprout on growth performance characteristics of WAD goats is presented in table 2. The result showed higher ($p<0.05$) effect on weight gain and metabolic weight gain for goats fed 30 mg/kgNC indicated that 30mg/kg NC is adequate for cellular uptake and translocation of the nutrient particles in the animal's body (Gatoo *et al.*, 2014) while the reduced body weight observed for 15 mg/kg NC could be based on the fact that lower doses of Cu-nanoparticles (Cu-NP) interacts more effectively with organic and inorganic materials due to their larger surface area (Zaboli *et al* 2013), and used for other body functioning rather than growth.

The result of levels of copper supplementation of fermented malted sorghum sprout on haematological parameters of WAD goats is shown in table 3. Eosinophil levels significantly ($p<0.05$) decreased with higher levels of copper supplementation while lymphocyte level significantly (p0.05) increased at higher levels of copper supplementation. The statistically lower neutrophil and basophil is an indication of immune system regulating activities of copper. According to (Weiss 1989), a defect in neutrophil activity might induce tissue injury through the production and release of reactive oxygen species (ROS). However, the significantly differed basophil and neutrophil for 15 mg/kg IC, 30 mg/kg IC, 15 mg/kg NC and 30 mg/kgNC were within the recorded range for clinically healthy WAD goats by (Merck 2011 and Daramola *et al.*, 2005). Meanwhile, the control groups had higher values than the normal range which might be linked to species and environmental differences. The significantly differed eosinophils and lymphocytes which were within the normal range for clinically healthy goats suggests that supplemented copper $CuSO_4$ and Cu-NP did not impair immune function of the experimental goats.

Table 2: Interactive effect of copper sources and different levels of dietary inorganic and nano copper supplementation of fermented malted sorghum sprout on growth performance characteristics of WAD goats

Parameters	0mg/ kg IC	15mg /kg IC	30mg /kg IC	0 mg /kg NC	15mg /kg NC	30 mg /kg NC	SEM
Initial weight (kg)	16.75	17.77	18.00	17.65	17.24	18.35	0.62
Final weight (kg)	20.75	19.63	21.89	21.65	19.13	22.65	0.71
Weight gain (kg)	4.00^{b}	1.86^{d}	3.89^{c}	4.00^{b}	1.89^{d}	4.30^{a}	0.366
Total dry matter intake (kg)	101.99	91.77	104.00	102.89	100.40	101.30	11.62
Feed conversion ratio	25.50^{d}	49.34^{b}	29.74^{c}	25.72^{d}	53.12^{a}	23.57^{e}	12.55
Metabolic weight (kgBW$^{0.75}$)	2.75^{b}	1.86^{d}	2.60^{c}	2.81^{b}	1.61^{e}	2.97^{a}	0.12

a,b,c,d,e Means with different superscripts in the same row are significantly (P<0.05) different
SEM: Standard Error of Mean. IC: inorganic copper, NC: nano copper

The result of levels of copper supplementation of fermented malted sorghum sprout on serum parameters of WAD goats is presented in table 4. Serum cholesterol and urea nitrogen were significantly (p<0.05) influenced by incremental levels of copper supplementation. Goats fed 0 mg/kg had the highest values for serum total cholesterol and serum urea nitrogen, while those offered 15 mg/kg and 30 mg/kg had the least values. Other serum parameters measured in were not significantly (p>0.05) influenced by the levels of copper supplementation. The significantly differed values for total cholesterol is an indication that the Cu levels had no stress implication on the experimental goats as serum cholesterol is an indicator for increasing levels of free radical damage in the body (Bansal and Jaswal 2009). The significantly differed values recorded for blood urea at 0mg/kg and 30mg/kg copper inclusion levels were higher than the normal values (Merck 2011). The increase might be attributed to environmental, species and nutritional differences.

Table 3: Main effect of levels of copper supplementation of fermented malted sprout on haematological parameters of WAD goats

Parameters	Reference value	0 mg/kg	15 mg/kg	30 mg/kg	SEM
Packed cell volume (%)	22-38	26.83	29.00	29.00	1.34
Haemoglobin (g/dl)	8-12	9.03	9.75	9.67	0.44
Red blood cell (x10$^{12/L}$)	8-18	15.77	16.12	16.07	0.22
White blood cell (x10$^{9/L}$)	4-13	7.42	7.22	8.28	0.49
Neutrophil (%)	30-48	36.67^{a}	31.67^{b}	32.50^{b}	1.44
Lymphocytes (%)	50-70	58.00^{b}	65.67^{a}	65.50^{a}	1.46
Eosinophil (%)	1-8	2.00^{a}	1.33^{a}	0.17^{b}	0.36
Basophil (%)	0-1	2.33^{a}	0.33^{b}	1.00^{b}	0.30
Monocyte (%)	0-4	1.00	1.00	0.83	0.45
Mean corpuscular volume (fl)	16-25	17.10	17.93	18.02	0.58
Mean corpuscular haemoglobin (pg)	5.2-8.0	5.70	6.03	6.00	0.19
Mean corpuscular haemoglobin concentration (g/dl)	30-36	33.43	33.63	33.33	0.12

[a,b] Means with different superscripts in the same row are significantly (P<0.05) different, SEM: Standard Error of Mean; Reference range by Merck (2011)

Table 4: Main effect of levels of copper supplementation of fermented malted sorghum sprouts on serum biochemical indices of WAD goats

Parameters	Reference value	0 mg/kg	15 mg/kg	30 mg/kg	SEM
Total protein (g/dl)	6.2-7.9	6.90	6.63	6.67	0.16
Albumin (g/dl)	2.9-4.3	4.07	3.95	3.98	0.14
Globulin (g/dl)	2.7-4.4	2.83	2.72	2.63	0.18
Glucose (mg/dl)	48-76	77.07	77.68	84.58	2.69
Creatinine (mg/dl)	0.6-1.6	1.53	1.37	1.52	0.12
Aspartate aminotransferase (u/l)	66-230	67.83	67.17	70.00	1.91
Alanine aminotransferase (u/l)	15-52	27.67	25.17	26.50	1.18
Total cholesterol (mg/dl)	65-136	110.80^{a}	89.97^{b}	91.55^{b}	6.01
Urea (mg/dl)	12-26	30.87^{a}	25.27^{b}	27.07^{b}	1.61

SEM: Standard Error of Mean; Reference range by Merck 2011

Table 5: Interactive effect of copper sources and different levels of dietary inorganic and nano copper supplementation of fermented malted sorghum sprout on oxidative stress biomarkers of WAD goats

Parameters	0 mg/kg IC	15 mg/kg IC	30 mg/kg IC	0 mg/kg NC	15 mg/kg NC	30 mg/kg NC	SEM
Catalase(U/g)	1.31^{ab}	1.16^{ab}	1.35^{a}	1.31^{ab}	1.18^{ab}	1.07^{b}	0.075
Glutathione(U/g)	233.00^{a}	229.57^{ab}	224.87^{ab}	233.00^{a}	231.27^{ab}	231.77^{ab}	2.29
Superoxide-dismutase (U/g)	1.89	1.91	1.87	1.80	2.14	1.80	0.13
Gualacol peroxidase (U/g)	7.17	6.90	7.03	7.17	6.93	6.97	0.23
Malondialdehyde (U/g)	1.80	1.50	1.50	1.80	1.42	1.53	0.18

SEM: Standard Error of Mean

The interactive effect of copper sources and different levels of dietary inorganic and nano copper on serum oxidative stress biomarkers of WAD goats is shown in Table 5. The serum oxidative biomarkers considered were not statistically different except for catalase and glutathione. The significantly differed catalase shows that higher level (30 mg/kgIC) of inorganic copper could aid in the dissociation of hydrogen peroxide to water and oxygen thereby protecting the cells from oxidative damage by reactive oxygen species (ROS). However, the comparable values of glutathione for the control, 0 mg/kgIC, 15 mg/kgIC, 0mg/kgNC, 15 mg/kgNC, was within the normal range for goats.

Interactive effect of copper sources and different levels of dietary inorganic and nano copper on serum minerals of WAD goats is presented in Table 6. The highest ($p<0.05$) calcium value was recorded for goats on 30 mg/kgIC and 15 mg/kgNC with comparable value for those on 15 mg/kgIC and 30 mg/kgNC while the least values were observed for goats offered 0 mg/kgIC and 0mg/kgNC. Goats fed 30 mg/kgIC and 15mg/kgNC had highest ($p<0.05$) value for copper concentration and least value for those on 0 mg/kgIC and 0 mg/kgNC while goats offered 15 mg/kgIC and 30mg/kgNC had similar values. Goats on 30 mg/kgIC had the highest value for manganese concentration which are similar with those fed 0 mg/kgIC, 0 mg/kgNC, 15 mg/kgIC, 15 mg/kgNC and 30 mg/kgNC. The significantly differed serum copper were within normal range 0.008mmol/l-0.099mmol/l reported by Ivan (1993). Copper content increased in the supplemented groups compared to the control. The incremental level of Cu also gave higher calcium level for supplemented groups; this shows the essentiality of copper normal physiological function (growth and bone) of the animal.

Table 6: Interactive effect of copper sources and different levels of dietary inorganic and nano copper supplementation of fermented malted sorghum sprout on serum minerals of WAD goats

Parameters	Reference value	0 mg/kg IC	15 mg/kg IC	30 mg/kg IC	0 mg/kg NC	15 mg/kg NC	30 mg/kg NC	SEM
Phosphorus (mmol/L)	+1.2-3.1	1.23	1.33	1.30	1.27	1.23	1.43	0.16
Sodium (mmol/L)	+137-152	81.20	72.40	88.50	81.20	92.73	77.27	10.15
Magnesium (mmol/L)	+0.9-1.2	1.27	1.37	1.30	1.23	1.23	1.17	0.09
Calcium (mmol/L)	+2.2-2.9	1.70^{b}	2.43^{ab}	3.10^{a}	1.70^{b}	3.10^{a}	2.43^{ab}	0.35
Potassium (mmol/L)	+3.8-5.7	5.50	5.53	5.73	5.50	6.00	5.47	0.34
Iron (mmol/L)	++0.023-0.025	0.22	0.22	0.21	0.22	0.22	0.22	0.008
Copper (mmol/L)	*0.008-0.099	0.06^{b}	0.07^{ab}	0.09^{a}	0.06^{b}	0.09^{a}	0.08^{ab}	0.005
Manganese (mmol/L)	**0.0001-0.0002	0.003^{ab}	0.003^{ab}	0.004^{a}	0.003^{ab}	0.003^{ab}	0.003^{ab}	0.000
Zinc (mmol/L)	#0.01- 0.019	0.08	0.08	0.07	0.08	0.08	0.08	0.004

^{a,b} Means with different superscripts in the same row are significantly (P<0.05) different; SEM: Standard Error of Mean; Reference range: +Merck 2011; ++Herdt and Hoff (2011); *Baumgartner (2009); **Pugh and Baird (2012); #Ivan (1993).

CONCLUSION

Dietary fermented malted sorghum sprout supplemented with 30 mg/kg nano copper have great potential to improve the productivity of West African dwarf goats. Dietary supplementation of 15mg/kg inorganic copper elevated Mean corpuscular haemoglobin concentration (MCHC) and lymphocytes level of the intensively raised WAD goats. Blood minerals (calcium and copper) were optimized by supplementation of dietary 30 mg/kg inorganic copper and 15 mg/kg nano copper.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest in the course of this research

REFERENCES

Aletor, V. A., Hamid, I. I., Nieb, E. and Pfeffer, E. 1998. Low-protein amino acids supplemented diets in Broilers. 1. Effects on growth performance, relative organ weight and carcass characteristics. In: *Proceedings of Silver Anniv. Conf. Nigerian Society of Animal Production* Pp. 153- 154.

AOAC, 2000. Official Methods of Analysis. 17th edition. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Aning, K. G., Ologun, A. G., Onifade, A., Alokan, J. A., Aletor, V. A. and Adekola, A. I. 1998. Effect of replacing dreid brewers' grain with sorghum rootlets on growth, nutrient utilization and some blood constituents in the rat. *Animal Feed Science and Technology* 71: 185-190.

Bansal, M. P. and Jaswal, S. 2009. Hypercholesterolemia induced oxidative stress is reduced in rats with diet enriched with supplement from dunaliella salina Algae. Am. *Journal of Biomedical Science*. 1(3): 196-204.

Baumgartner, W. 2009: Clinical Propaedeutics of House Animals and Pets (in German). 7th edition. Parey, Stuttgart. xiv+525 p.

Belton P. S. and Taylor J. R. N 2004. Sorghum and millets: Protein sources for Africa. Trends *Food Science Technology*. 15(2): 94-98.

Daramola, J. O., Adeloye, A. A., Fatoba, T. A. and Soladoye, A. O. 2005. Haematological and biochemical parameters of West African Dwarf goats. *Livestock Research for Rural Development 17*(8): 3.

Fanimo, A.O. and Akinola, O.S. 2006. Response of broiler chickens to raw and processed malted sorghum sprout. *European Symposium on Poultry Nutrition*. Held Hungary.

Gatoo M. A., Naseem, S., Arfat, M. Y., Mahmood Dar, A., Qasim, K. and Zubair S. Physicochemical properties of nanomaterials: implication in associated toxic manifestations. *Biomedical Result. International*. 2014, 1–8.

Herdt, T. H., Hoff, B. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. The Veterinary Clinics of North America: *Food Animal Practice* 27, 255–283.

Merck Manul. 2012. Haematological reference ranges. Merk Veterinary Manual. Retrieved from http://www.merckmanuals.com/.

Ososanya, T. O., Alabi, B. O. and Sorunke, A. O. 2013. Performance and digestibility of corncob and cowpea husk diets by West African dwarf sheep. *Pakistan Journal. Nutrition*., 12 (1): 85-88.

Pugh, D. G and Baird, A. N. 2012. Sheep and Goat edicine. 2nd edition. Saunders Elsevie, Maryland Heights. xiv+