



ORIGINAL RESEARCH ARTICLE

Influence of fermented malted sorghum sprout based diet supplemented with copper glycine on oxidative stability in West African dwarf goats

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ABSTRACT

A 90-day study was conducted to determine the influence of fermented malted sorghum sprout (FMSP) based diet supplemented with copper glycine (Cu-G) on oxidative stability of West African dwarf (WAD) goats. A total of twenty-four (24) WAD goats weighing 6.00 ± 0.2 kg were allotted to four dietary treatments (0 mg/kg, 10 mg/kg, 20 mg/kg, and 30 mg/kg Cu-G, for treatment 1, 2, 3 and 4 respectively) in a completely randomized design. Fastened blood samples were collected to assay for oxidative stability; thiobarbituric acid reactive substance (TBARS), total antioxidant activity (TAA $\mu\text{mol/L}$), glutathione (GSH $\mu\text{mol/L}$), superoxide dismutase (SOD ng/mL) and catalase (CAT u/mL) using standard procedures. Serum TBARS (0.65-0.81 $\mu\text{mol/L}$) values decreased ($P < 0.05$) across the dietary treatments as the Cu-G supplementation increased. Serum TAA values were significantly ($P < 0.05$) increased across the dietary treatments as the Cu-G supplementation increased (49.16 to 55.36 $\mu\text{mol/L}$). Significant ($P < 0.05$) difference among variables were observed in the GSH values (49.39-53.17 $\mu\text{mol/L}$), SOD (38.55-49.87 ng/mL) and CAT (180.97-200.59 u/mL). The serum TAA, GSH, SOD and CAT values observed increased ($P < 0.05$) with increasing Cu-G supplementation. It can therefore be concluded that, the reduced TBARS values and increased values of antioxidants with Cu-G inclusion is an indication that copper ensure oxidative stability condition in WAD goats fed 30 mg/kg inclusion level.

Keyword: By-products, trace minerals, antioxidant, ruminant, free radicals.

INTRODUCTION

Agro-industrial by-products can be used as part of livestock feed as it reduces the cost of feeding, improve animals' production potential and facilitates farmers profitability (Abarar *et al.*, 2002). They also eliminate the competition between humans and animals for the scarce feed resources. Malt is extracted from the germinated sorghum seeds and the residue consists of sorghum shoots and roots that are referred to as malted sorghum sprouts (MSP) (Aletor *et al.*, 1998). MSP is a by-product of malt processing companies (Ikediobi *et al.*, 1989) with potentials as feed stuff for ruminants (Ologun *et al.*, 1998). Processing methods including soaking, sprouting and fermentation have been reported to improve the nutritional and functional properties of plant seeds (Jirapa *et al.*, 2001). Fermented MSP enhances productivity in West African Dwarf

goats (Saka *et al.*, 2016). Previous studies revealed that among the trace minerals present in MSP, copper was deficient while zinc was the most abundant (Aning *et al.*, 1998). Meanwhile, the importance of Copper (Cu) in ruminant nutrition cannot be waved aside, it is an essential trace mineral required for proper functioning of the central nervous system, immune system, cardiovascular system, pigmentation of the skin (Close, 1998) and other various enzymatic systems in the body (Lim and Paik, 2006). Copper being an antioxidant agent helps to prevent against oxidative stress (Miller *et al.*, 1979) which results from increased production of free radicals, reactive oxygen species (ROS) with a decrease in antioxidant defence (Trevisan *et al.*, 2001). The evaluation of oxidative stress is very important in ruminant health and animal production as complementary tool in evaluation of the nutritional

and metabolic status of the animal. (MohebbiFani *et al.*, 2012). However, the relevance of antioxidant in living organisms cannot be over emphasized (Chance *et al.*, 1979) as it acts in numerous ways that include the prevention of oxidative stress by scavenging free radicals and quenching reactive oxygen species (Dunnet, 2003) as well as maintaining their immunity level. Therefore, this experiment aimed to assess the influence of fermented malted sorghum sprout supplemented with copper glycine (Cu-G) on thermo-physiological parameters and oxidative stability of West African dwarf goats fed.

MATERIALS AND METHODS

Site Description

The experiment was conducted at the Teaching and Research Farm of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo-state in the South-Western part of Nigeria. The area lies within the rain forest ecological zone, and falls within longitude and latitude 7°27' and 3° 25' respectively at altitude 200- 300m above the sea level with an annual rainfall of about 1250mm. The temperature and relative humidity ranges from 30 – 35°C and 76 – 84% respectively.

Source and Processing of Test Ingredient

The test ingredients used were malted sorghum sprout (MSP) and copper glycine (Cu-G). The MSP was purchased in dried form from an agro-allied industry at Ijoko Ota, Ogun State, Nigeria and the Cu-G was purchased from Wisium Nutrition Firm Service, International Premix Brand while Copper free premix was purchased from Rotinol Company, Lagos State, Nigeria. MSP was fermented using the procedure of Fanimu and Akinola (2006). Fermentation involved the use of water and polythene bag. Water was mixed with dried MSP at a ratio of 1:2 (1 litre of water to 2g of MSP) so that the entire sprout moistens. The mixture was then transferred into the air-tight polythene bag and fermented under room temperature for 72 hours. Thereafter, fermented malted sorghum sprout (FMSP) was spread on concrete floor for sun drying. After drying, Cu-G at varying levels of 0, 10, 20 and 30mg/kg was incorporated into 30% fermented malted sorghum sprout (FMSP) to formulate four experimental diets as indicated in Table 1. Each

preparation was then mixed with other ingredients as contained in Table 1.

Experimental Animals, Management and Design

Twenty four (24) West African Dwarf (WAD) goats averaged of $6.00 \pm 0.2\text{kg}$ were used in a 90-day research trial. The animals were housed intensively in a well-ventilated individual pen, disinfected with Izal solution two weeks prior to the experiment. On arrival, the goats were secluded for 28 days and during this period: they were given prophylactic treatments consisting of intra-muscular injection of oxytetracycline LA (1ml/10kg BW) and vitamin B complex. They were also routinely dewormed with 1ml/10kg BW of Albendazole and subsequently injected with 0.5ml/10kg BW of ivermectin to eliminate both internal and external parasites respectively. Homologous *Pestes des petits ruminant* (PPR) vaccine was administered against PPR disease to make sure that the goats were in good condition. The animals were adapted to pen environment for 14 days and fresh cool clean water was also supplied *ad libitum*. During the pen adaptation period, the experimental animals were balanced on weight equalization into four treatment groups, during which they were introduced to experimental diets (control diet) and the basal ration (*Panicum maximum*). The goats were fed 5% of their body weight consisting of 3% experimental diet and the remaining 2% basal ration. The feed was offered at 07:30am and grasses were given in the afternoon at exactly 1:00pm daily. The twenty four West African Dwarf (WAD) goats were divided into four treatment groups of six (6) goats in a group and were randomly allocated on weight equalization to four dietary concentrates in a completely randomized design.

Chemical Analysis

Subsamples of feed offered were ground to pass 1mm sieve screen using laboratory hammer mill and analyzed for crude protein, ether extract and ash content (AOAC, 2002). Fibre fractions; neutral detergent fibre (NDF), Acid detergent lignin (ADL) were determined according to the procedure of Van soest *et al.* (1991).

Oxidative stability indicators

Blood samples (3mL) were collected via the jugular vein from all animals using hypodermic needle and syringe and they were released into plain bottles. Serum was then obtained using standard procedures and assayed for peroxidation, antioxidant activity, catalase and superoxide dismutase. Serum total antioxidant activity was carried out according to Koracevic (2001), Superoxide dismutase (SOD) was estimated by the method of Marklund and Marklund (1974) as modified by Soon and Tan (2002) and Catalase was estimated by Beers and Sizer (1952) method,

Serum lipid peroxidation was determined using thiobarbituric acid assay according to Ohkawa *et al.*(1979).

Statistical Analysis

Data obtained in this study were subjected to one way analysis of variance (ANOVA) using statistical analysis software SAS (2005) while significant means were separated at 5% level of significant using Turkey's test of the same statistical package.

Table 1: Gross Composition of the Experimental Diet

Ingredients (g/kg)	Inclusion levels of Copper glycine (mg/kg)			
	0	10	20	30
Maize bran	400.00	400.00	400.00	400.00
Wheat offal	242.5	242.5	242.5	242.5
Fermented Malted Sorghum Sprout	300.00	300.00	300.00	300.00
Premix(Copper free)	2.50	2.50	2.50	2.50
Limestone	50.00	50.00	50.00	50.00
Salt	5.00	5.00	5.00	5.00
Copper glycine (Cu-G)	-	++	+++	++++
Total	1000.00	1000.00	1000.00	1000.00
Determined Analysis (g/kg)				
Dry matter	863.00	864.00	868.80	857.80
Crude protein	161.7	148.60	141.90	153.80
Ether extract	25.40	30.10	30.10	29.30
Ash	109.10	105.90	117.10	98.80
Non fibre carbohydrate	423.70	385.40	357.90	354.80
Organic matter	890.80	894.10	882.90	901.20
Neutral detergent fibre	280.00	330.00	353.30	363.30
Acid detergent fibre	66.70	53.30	70.00	48.70
Acid detergent lignin	26.70	20.00	30.00	16.70
Cellulose	40.00	33.30	40.00	30.00
Hemicelluloses	213.30	276.70	283.30	316.70

Cu-G: Copper Glycine, - : 0mg/kg Cu-G level, ++ :10mg/kg Cu-G inclusion level, +++ :20mg/kg Cu-G inclusion level, ++++: 30mg/kg Copper glycine inclusion level.

RESULTS

Table 2 represents the oxidative stress indicators of West African Dwarf Goats fed fermented malted sorghum sprout supplemented with varying

levels of copper glycine (Cu-G). All parameters considered were significantly ($P<0.05$) influenced by the Cu-G inclusion levels. The values of thiobarbituric acid reactive substance (TBARS)

decreased ($P<0.05$) across the dietary treatments as the inclusion level of Cu-G supplementation increased. The TBARS concentration ranged from 0.66-0.81 $\mu\text{mol/L}$ with buck fed diet containing 0mg/kg Cu-G inclusion level having the highest (0.184 $\mu\text{mol/L}$) values while those fed diet containing 30mg/kg Cu-G recorded the lowest (0.655) values. The total antioxidant activity (TAA) values obtained in this study ranged from 49.10- 55.36 $\mu\text{mol/L}$. The TAA values observed in this study increased across the dietary treatments as the inclusion of Cu-G increased in which goats fed diet supplemented with 30mg/kg Cu-G

recorded the highest values while the least values was observed in goats on 0mg/kg Cu-G supplemental diet. The SOD values obtained in this study was significantly ($P<0.05$) increased from 38.55ng/ml in goats fed 0mg/kg Cu-G to 49.87ng/ml in goats fed 30mg/kg Cu-G supplemental diet. The Glutathione (GSH) values was highest (53.17 $\mu\text{mol/L}$) in goats fed 30mg/kg Cu-G supplemental diet. Goats on Cu-G supplementation groups recorded similar catalase (CAT) values but significantly higher than those on the control diet.

Table 2: Oxidative stability of West African dwarf goats fed fermented malted sprout supplemented with varying levels of copper glycine

Parameters	Inclusion levels of Copper Glycine (mg/kg)				SEM
	0	10	20	30	
TBARS ($\mu\text{mol/L}$)	0.814 ^a	0.68 ^b	0.67 ^b	0.655 ^b	0.021
TAA ($\mu\text{mol/L}$)	49.10 ^b	51.29 ^{ab}	52.49 ^{ab}	55.36 ^a	1.00
GSH ($\mu\text{mol/L}$)	51.53 ^{ab}	50.90 ^{ab}	49.39 ^b	53.17 ^a	0.61
SOD (ng/mL)	38.55 ^b	40.94 ^b	47.25 ^a	49.87 ^a	1.50
CAT (u/mL)	180.97 ^b	197.09 ^a	195.18 ^a	200.59 ^a	2.73

^{a, b} Means along the same row with different superscripts are significantly different ($p<0.05$) TBARS: Thiobarbituric acid reactive substance, GSH: Glutathione, TAA: Total antioxidant activity, SOD – Superoxide dismutase, CAT – Catalase,

DISCUSSION

Antioxidant can be broadly defined as any substance that delays, prevents or remove oxidative damage from a target molecule (Halliwell and Gutteridge, 1995). These antioxidants can be broadly divided into enzymatic (Superoxide dismutase: SOD, Glutathione peroxidase: GSHPx and Catalase: CAT etc) and non-enzymatic (Vitamin E and Selenium etc). GSH is a non-enzymatic antioxidant in both plant and animal which is capable of preventing damage to important cellular component caused by reactive oxygen species (ROS) such as free-radicals, peroxide, lipid peroxide and heavy metals (Pompella *et al.*, 2003). The role of copper in reducing oxidative stress in livestock animals has been well documented (Miller *et al.*, 1979; Kleczkowski *et al.*, 2003). This is reflected in Thiobarbituric acid reactive substance (TBARS) reducing capacity of the dietary Cu-G supplementation. Atlan *et al.* (2003) demonstrated that lipid peroxidation is associated with the

production of large number of free radicals and can be used for evaluation of oxidative status severity (Halliwell and Whiteman, 2004). On this note, this indicated that Cu-G supplementation was able to curb the activities of free radical to cause lipid peroxidation in the experimental goats. The importance of TAA as an instrument to estimate the relationship between diet and oxidative stress has been reported by the studies of Serafini *et al.* (2002) and Brighenti *et al.* (2005). Wayner *et al.* (1987) also reported that TAA creates a dynamic equilibrium that is influenced by the interaction between each serum antioxidant constituent hence the cooperation of antioxidant in serum provides protection against free radicals than any other antioxidant alone. However, the increased Total antioxidant activity (TAA) values observed in this study can be attributed to the role of copper in antioxidant production as reported by Nockel (1994). It can be inferred from this study, therefore, that 30mg/kg supplementation was able to create a high antioxidant capacity by scavenging excess free radicals. Summarily, the

increase inclusion level of Cu-G, increased the GSH level, thus facilitating the entering of copper into the cell (Maryon *et al.*, 2013) and its increase significantly increases the rate of copper intake which makes copper available in the system as reported by Maryon *et al.* (2013). The increase in the level of GSH also influenced the level of other enzymatic antioxidant (SOD and CAT) as the Cu-G inclusion level increased across the dietary treatments. Mohammed *et al.* (2015) reported that SOD (one of the enzymatic antioxidant) is the first line of defence and its role is to accelerate the dismutation of toxic superoxide produced during oxidative energy process to hydrogen peroxide, therefore its increase as the inclusion level of Cu-G increased can be attributed to the function of copper in contributing to various enzymes like SOD as they exhibit their roles. A reduction in SOD leads to oxidative damage of phagocytes therefore reducing their effectiveness in engulfing and removing pathogens in the system (Suttle and Jones, 1989). This is because SOD has been found to influence phagocyte (immune cell) function in ruminant (Xin *et al.*, 1991). SOD and GSH are regarded as chain breaking antioxidant (Ko *et al.*, 2005; Kokoglu *et al.*, 2012), whose function is to receive an electron or donate an electron to a radical forming stable products (Halliwell, 1995). A classic example of such chain reaction is lipid peroxidation, and the reaction will continue to propagate until two radicals combine to form a stable product or the radicals are neutralized by a chain breaking antioxidant (De Zwart, 1999), such can be related to the reduced TBARS and increased total antioxidant capacity of the selected biomarkers. An increase in CAT values observed was influenced by increase in SOD values emanated as a result of increase in the level of Cu-G supplementation, thus, hydrogen peroxide being an end product of superoxide ion dismutation would have been converted to water by CAT alongside with another enzymatic antioxidant Glutathione Peroxidase (GSH-Px) (Berr *et al.*, 2004; Bourdel-Marchasson *et al.*, 2001). In animals, hydrogen peroxide is detoxified by CAT and GPx. CAT has been reported to protect cell from hydrogen peroxide generated within them and plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Suffice to say, a balance between both the activities and intracellular levels

of this antioxidant are essential for the survival of organisms and their health (Maydani, 2001) through protective antioxidant systems.

CONCLUSION

Conclusively, the reduced values of thiobarbituric reactive acid substances with associated increase in SOD, GSH and TAA with Copper-Glycine inclusion is an indication of oxidative stability in West African Dwarf goats fed between 10-30mg/kg inclusion level incorporated in fermented malted sorghum sprout.

CONFLICT OF INTEREST

Authors hereby declare that there is no conflict of interest as far the conduct of this study is concerned.

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