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Performance and Oxidative Stress Biomarkers of West African Dwarf Goats Fed Diet Containing Incremental Sodium Humate

Research Article

T.T. Ikyume[1,2*], A.O. Yusuf[2], A.O. Oni[3], O.S. Sowande[2], S. Ikuejamoye-Omotore[2] and S.S. Dansu[2]

[1] Department of Animal Production, Federal University of Agriculture, Makurdi, Nigeria
[2] Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria
[3] Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Nigeria

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*Correspondence E-mail: ikyumett@uam.edu.ng

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ABSTRACT

Problems relating to reduced performance owing to mineral deficiencies in grazing animals and oxidative stress occasioned by changing weather condition can be addressed through strategic mineral supplementation and improving antioxidant capacity of the animals. A 97-day trial was designed to assess the performance and apparent nutrient digestibility of West African Dwarf (WAD) goats fed diets with incremental levels of sodium humate. Thirty (30) WAD bucks of ages between 10-15 months were used for this study. The bucks were randomly assigned to five dietary treatments containing 0, 5, 7.5, 10 and 12.5 g/kg diet of sodium humate laid out as completely randomized design. Data on weight changes, dry matter concentrate intake, feed conversion ratio (FCR), nutrient digestibility and rate of mineral absorption were obtained and statistically analysed using the generalized linear model (GLM) of Statistical Package for Social Sciences (SPSS) (version 23). Results revealed that sodium humate supplementation improved ($P<0.05$) weight gain, FCR, mineral (Zn, Cu, Mn and Na) absorption and nutrient (dry matter (DM), crude protein (CP), crude fibre (CF), ash, ether extracts (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF)) digestibility. Supplementation of sodium humate decreased concentrate intake ($P<0.05$). After 30 days of sodium humate supplementation, malondialdehyde (MDA) increased ($P<0.05$) at higher levels, glutathione peroxidase also increased up to the level of 7.5 g/kg diet sodium humate and then decreased in subsequent levels, with superoxide dismutase (SOD) observed to decrease ($P<0.05$). However, at 90 days of sodium humate supplementation, MDA, nitric oxide (NO) and bilirubin decreased ($P<0.05$) while albumin and uric acid increased ($P<0.05$).Conclusively, up to 12.5 g/kg diet supplementation of sodium humate sufficiently enhanced performance of WAD goats while also improving their antioxidant capacity.

KEY WORDS digestibility, goats, growth performance, oxidative stress, sodium humate.

INTRODUCTION

The West African Dwarf (WAD) goats are said to be well adapted to the agro-ecological conditions, but not under all production systems (Yusuf *et al*. 2017). The semi-intensive system is reported to be the most common in greater parts of humid tropics and that, it is touted to have high production potential with less labour input compared to the extensive and intensive systems (Ajala *et al*. 2008). The rearing of these goats under semi-intensive system of management also creates a balance between animal welfare and productivity of the animals. Nevertheless, there are problems relating to the anticipation of poor production owing to mineral deficiencies and other associated factors (heat stress, physi-

cal stress, and worm infestation) in grazing livestock (Suttle and Jones, 1988; Maurya *et al*. 2012; Kumar *et al*. 2013).

The nutrient quality of forage, chiefly perennial warm-season grasses, is repeatedly lacking in trace mineral content to meet the requirements of grazing animals (Arthington, 2017). In addition, the changing weather condition occasioned by global warming has remained a challenge for these grazing animals. This has led to incidences of heat stress in the animals thereby, affecting their performance. Yusuf *et al.* (2017) reported that while antioxidant capacity of semi-intensively managed WAD goats were higher at the start of rainy season (May-June) in South west Nigeria, they were observed to decrease at late rainy season (September-October). To enhance performance therefore, strategies employed should ensure supplementation of feed additives that will improve the antioxidant capacity of these animals and improve the mineral absorption rates of the animals.

Sodium humate or humic substance as an organic substance does not only have the capacity to repudiate oxidative stress, it can also supply trace minerals that are required for optimum productivity of grazing animals. There is increased interest in the use of humates as a feed additive, with proof that they may have beneficial effects on digestion, growth and the immune system in poultry, swine, goats, sheep and cattle (Islam *et al.* 2005; Ji *et al.* 2006).

The composition of humates includes humus, humic acid (HA), fulvic acid, ulmic acid, and trace minerals, which are necessary for development (Kocabagli *et al*. 2002). Scott (1998) has also reported that quinone groups are primarily organic radicals in humates, which act as a terminal electron acceptor for organic substrates and the oxidation of hydrogen. This action of the quinone group makes humate useful even in reducing the competition for hydrogen by methanogens. In suckling calves, humate was used to check oxidative stress in order to improve their health (Ipek *et al*. 2008; Weber *et al*. 2014). The effect of humates on nutrient digestibility, methane emissions, and rumen micro biota in beef heifers has also been reported (Terry *et al.* 2018). Sodium humateis reported to stabilize pH of rumen and improve fungi populations in goats (Ikyume *et al.* 2020a). It is worthy to note that much of the reports on the use of humic acids (HA) and its sodium salt (HNa) has been on dairy and beef cow, pig and poultry. Also, its effect on rumen environment in grazing WAD goats has been reported, but not much has been reported on its antioxidant capacity to handle stress from management system and changing climate in small ruminant production systems especially for West African Dwarf goats. Therefore, this research assessed the effect sodium humate supplemented diet on growth performance, trace mineral absorption, apparent nutrient digestibility and oxidative stress biomarkers of semi- intensively managed West African Dwarf (WAD) goats.

MATERIALS AND METHODS

Experimental site

The feeding trial will be carried out at the Small Ruminant Experimental Unit of Directorate of University Farms (DUFARMS), Federal University of Agriculture Abeokuta Nigeria. Abeokuta is located in the rainforest vegetation Zone of the South-Western Nigeria on Latitude 7° 13' 49.46'' N, longitude 3° 26' 11.98''E and an altitude 76 m above sea Level (Google Earth 2015). The climate is humid with a mean annual rain fall of 1037 mm and annual mean temperature and humidity of 43.7 °C and 82 respectively (Meteorology Department, Ogun – Osun River Basin Authority, Abeokuta, Ogun State, Nigeria).

Ingredient collection and formulation of diet

Sodium humates was obtained from a reputable Company in China. Other feedstuff used in formulating experimental diet such as maize, wheat offal, palm kernel cake, bone meal, mineral premix and salt were purchased from a feed shop in Abeokuta, Ogun state. The ingredients were milled into coarse form and mixed together to form concentrate diet to contain 0, 5, 7.5, 10 and 12.5 g/kg diet of sodium humates, respectively (Table 1). The recommendation of 5 g/kg diet for sheep (Covington *et al*. 1997) informed the basis for choosing these levels of inclusion in goats.

Experimental design, animal management and diet

A total of thirty (30) West African Dwarf bucks aged between 10-15 months, with mean weight of 7.19 ± 0.83 kg were purchased from local farmers from Abeokuta and environs. Oxytetracycline LA (1 mL/10 kg) was administered to the animals for prophylactic treatment against bacterial disease while Ivemectin LA (1 mL/50 kg) was administered against internal and external parasite infestation. The animals were allotted to a 1 m^2 pen, meanwhile, they were allowed to graze together 6 hrs daily (9:00 a.m.–3.00 p.m.) within a confined area containing sown *Panicum maximun*. On return, they were supplemented concentrate (experimental diet) at 4% of their body weight. Water was provided *ad-libitum.* Experimental and animal management procedures were as approved by Ethics Committee of College of Animal Science and Livestock production, Federal University of Agriculture, Abeokuta, Nigeria (ethical clearance number COLANIM/APH/PG/14/0107). The experimental design was a completely randomized design and the experiment lasted for a period of 97 days comprising of 90 days feeding trial and 7 days of digestibility trial.

Table 1 Ingredient and chemical composition of experimental diets

Ingredient (%)	Experimental diets				
	Control	5HNa	7.5HNa	10HNa	12.5HNa
Maize offal	30	30	30	30	30
Wheat offal	34	34	34	34	34
Palm kernel cake	32	32	32	32	32
Bone meal	3	3	3	3	3
Vitamin premix	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
HNa[1]	-	0.5	0.75	1	1.25
Total	100	100	100	100	100
Chemical composition (%)					
Dry matter (DM)	88.00	88.00	88.00	88.00	89.00
Crude protein (CP)	14.88	14.01	14.35	14.13	14.61
Crude fibre (CF)	9.50	9.50	10.00	9.00	10.00
Ash	5.00	5.40	5.45	6.00	6.50
Ether extracts (EE)	6.50	8.00	7.50	8.00	7.56
Neutral detergent fiber (NDF)	64.00	65.00	63.00	54.00	55.00
Acid detergent fiber (ADF)	22.00	23.00	19.00	23.00	20.00
Acid detergent fiber (ADL)	9.00	8.50	8.00	9.00	7.00

[1] HNa: Sodium humate does not add up in the composition of experimental diets.
Control: 0 g/kg diet sodium humate inclusion; 5HNa: 5 g/kg diet sodium humate inclusion; 7.5HNa: 7.5 g/kg diet sodium humate inclusion; 10HNa: 10 g/kg diet sodium humate inclusion and 12.5HNa: 12.5 g/kg diet sodium humate inclusion.

The treatment diets were as follow: 1) control (0 g/kg diet of sodium humate supplementation); 2. 5HNa (5 g/kg diet of sodium humate supplementation); 3. 7.5HNa (7.5 g/kg diet of sodium humate supplementation); 4. 10HNa (10 g/kg diet of sodium humate supplementation); and 5. 12.5HNa (12.5 g/kg diet of sodium humate supplementation).

Data collection

The temperature and relative humidity of the environment for the three months of rearing the animals was recorded twice daily (at 7.00 a.m. and 1.00 p.m.) in the morning and afternoon. The records of temperature and relative humidity were used to calculate the temperature-humidity index (THI) of the environment using the following equation (NRC, 1971):

$$THI = (1.8\times T+32) - (0.55-0.0055\times RH) \times (1.8\times T-26)$$

Where:
T: air temperature (˚C).
R: relative humidity (%).

The initial body weight of the WAD bucks was determined at commencement of the experiment with the aid of a digital weighing scale. A change in weight of the goats was obtained on weekly basis. Concentrate intake was determined on a daily basis. Feed conversion ratio was calculated as the ratio of feed intake and weight gain.

The animals were moved into metabolic cages for feacal collection.

They were allowed four days to acclimatize; thereafter feacal samples were collected from each of the animals for three days on a daily basis in the morning and afternoon. Faeces were collected from wire-mesh nets placed under the floor of metabolism crates. Collected feacal samples were weighed daily then oven dried at 65 ˚C for 48 hrs, then ground to pass through a 1-mm screen and bulked on replicate basis, and stored in air tight container for chemical analysis. Samples of the experimental diet and the feacal samples collected during digestibility trial were analyzed for mineral digestibility. Albumin, bilirubin, uric acid, superoxide dismutase (SOD), glutathione peroxidase (GSHPx), nitric oxide (NO), malondialdehyde (MDA) were determined three times during the experiment (30, 60 and 90 days respectively). Serum albumin concentration was determined as described by Maier *et al*. (2007) and Shin *et al*. (2009). Bilirubin was assayed as described by Nedredal *et al.* (2009) and Beppu *et al.* (2009). Glutathione peroxidase was spectrophotometrically analyzed as described by Chen *et al*. (2014). Malondialdehyde (MDA) was assayed as described by Davison (2011). Superoxide dismutase (SOD) activity in blood serum was determined by the method of Janknegt *et al.* (2007). Then itricoxide (NO) level from the parameters of antioxidant in the blood serums was spectrophotometrically analysed as described by Zeng *et al*. (2011). Uric acid was analysed as described by Whidden *et al*. (2009).

Table 2 Temperature-humidity index (THI) (%) of the rearing environment for the duration of experiment

Month	Morning	Afternoon
June	74.76	82.73
July	74.34	78.76
August	73.96	78.98

Experimental diets and feacal samples were analyzed for dry matter (DM) crude protein (CP), crude fibre (CF), ash, ether extract (EE) by procedure of AOAC (2005), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analyzed as outlined by Van Soest *et al.* (1991).

Procedure of Larrauri *et al.* (1996) was adopted to measure the minerals in the feed and feaces. To measure the mineral content, the ash was dissolved in HNO_3 with 50 g/L of $LaCl_3$and the various mineral components were analyzed separately, using an atomic absorption spectrophotometer (AAS).

Statistical analysis

The weight gain, concentrate intake, feed conversion ratio, apparent nutrient digestibility parameters, rate of absorption of the various trace minerals and oxidative stress biomarkers were analysed using one-way analysis of variance as contained in the general linear models procedures of SPSS (2011). Significant differences among treatment means where applicable were separated using the generalized linear model (GLM) procedure of SPSS. Probability significance was declared at $P \leq 0.05$. The statistical model adopted is as follow:

$$Y_{ij} = \mu + T_i + £_{ij}$$

Where:

Y_{ij}: observed value of the dependent variables (weight gain, concentrate intake, feed conversion ratio, apparent nutrient digestibility parameters, rate of absorption of the various trace minerals and oxidative stress biomarkers).

μ: population mean.

T_i: effect due to level (0, 5, 7.5, 10 and 12.5 g/kg diet) of inclusion of HNa.

$£_{ij}$: random residual value.

RESULTS AND DISCUSSION

The result of the temperature-humidity index (THI) for three months duration of rearing is presented at Table 2. The THI was highest for the month of June for both morning and afternoon periods (74.76 and 82.73, respectively). These were however lower in the month of July and August. The THI for the month of July was 74.34 and 78.76 (morning and afternoon) while that of August was 73.96 and 78.98 (morning and afternoon).

Table 3 shows the growth performance characteristics of semi-intensively managed West African Dwarf (WAD) goats on diet containing incremental levels of sodium humate. Daily weight gain had comparable higher ($P<0.05$) values in 5HNa 7.5HNa, 10HNa and 12.5HNa groups and this linearly decreased ($P<0.05$) in the control group. Although a quadratic responses among the treatment was observed in the 12.5HNa group. Least means of the daily weight gain were observed in control group. Concentrate intake also had a linear and quadratic relationship across the treatment groups. Highest ($P<0.05$) daily concentrate intake was found in the control group. All sodium humate supplemented groups had similar ($P>0.05$) values for daily concentrate intake. Feed conversion ratio (FCR) was better in all the sodium humate supplemented groups but reduced ($P<0.05$) in the control group.

Table 4 shows the result of apparent nutrient digestibility of WAD goat on concentrate diet containing incremental levels of sodium humate. Dry matter digestibility was highest ($P<0.05$) in 7.5HNa group and quadratically decreased ($P<0.05$) in other groups with least DM in control group. Crude protein (CP) digestibility was linearly increased ($P<0.05$) in sodium humate supplemented groups. The CP digestibility was highest ($P<0.05$) in 7.5HNa group and this reduced ($P<0.05$) in the other groups with the control group having the least crude protein digestibility. Crude fibre digestibility had higher ($P<0.05$) similar digestibility coefficient values in the 5HNa and 7.5HNa groups but linearly decreased in control, 10HNa and 12.5HNa which had least similar values. Ash digestibility linearly increased ($P<0.05$) with 10HNa and 12.5HNa groups having higher similar values and this reduced ($P<0.05$) in the control, 5HNa and 7.5HNa groups which had least similar values. Ether extract was highest ($P<0.05$) in 7.5HNa group and reduced ($P<0.05$) in the other groups, with similar least values obtained in control, 5HNa and 12.5HNa groups. Cubic relationship was observed among the various treatments for neutral detergent digestibility (NDF). Higher ($P<0.05$) similar coefficients of NDF digestibility were observed in control, 5HNa, 7.5HNa and 10HNa groups but reduced ($P<0.05$) in the 12.5HNa group. For ADF digestibility, higher ($P<0.05$) similar values were observed in 7.5HNa, 10HNa and 12.5HNa groups, but decreased in the control and 5HNa groups with least value observed in the control group. Acid detergent lignin coefficient of digestibility was not affected by feeding of incremental levels of sodium humate.

Table 3 Growth performance of semi-intensively raised West African Dwarf (WAD) goats supplemented concentrate diets containing varying levels of sodium humate

Growth performance	Treatment groups					SEM	Polynomial contrast		
	Control	5HNa	7.5HNa	10HNa	12.5HNa		L	Q	C
Initial weight (kg)	7.04	7.25	7.22	7.21	7.22	0.15	NS	NS	NS
Final weight (kg)	9.37	10.35	10.38	10.57	10.08	0.17	NS	NS	NS
Weight gain (kg)	2.33^{b}	3.10^{a}	3.15^{a}	3.36^{a}	2.86^{ab}	0.09	**	***	NS
Daily weight gain (g)	25.85^{b}	34.44^{a}	35.02^{a}	37.33^{a}	31.72^{ab}	0.98	**	***	NS
Daily concentrate intake (g)	129.54^{a}	93.84^{b}	88.59^{b}	96.45^{b}	94.67^{b}	3.87	**	**	NS
Feed conversion ratio	5.10^{a}	2.75^{b}	2.51^{b}	2.59^{b}	2.99^{b}	0.21	***	***	*

Control: 0 g/kg diet sodium humate inclusion; 5HNa: 5 g/kg diet sodium humate inclusion; 7.5HNa: 7.5 g/kg diet sodium humate inclusion; 10HNa: 10 g/kg diet sodium humate inclusion and 12.5HNa: 12.5 g/kg diet sodium humate inclusion.
L: linear; Q: quadratic and C: cubic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
NS: non significant.

Table 4 Apparent nutrient digestibility (%) of West African Dwarf (WAD) goats fed concentrate containing incremental levels of sodium humate

Nutrient	Treatment groups					SEM	Polynomial contrast		
	Control	5HNa	7.5HNa	10HNa	12.5HNa		L	Q	C
Dry matter	76.26^{d}	88.83^{a}	91.44^{a}	84.44^{b}	81.16^{c}	1.04	NS	***	***
Crude protein	77.56^{c}	87.47^{a}	89.29^{a}	84.05^{b}	82.14^{b}	0.80	**	***	***
Crude fibre	56.11^{bc}	64.95^{a}	59.15^{ab}	51.68^{c}	52.46^{bc}	1.16	**	NS	***
Ash	27.81^{b}	33.36^{b}	32.91^{b}	52.86^{a}	45.46^{a}	1.90	***	***	***
Ether extract	79.89^{c}	81.37^{c}	89.33^{a}	86.14^{b}	81.67^{c}	0.72	**	***	***
NDF	62.29^{ab}	63.86^{ab}	65.47^{a}	64.13^{a}	56.34^{b}	0.98	NS	**	NS
ADF	41.46^{c}	43.47^{bc}	50.43^{ab}	55.98^{a}	48.71^{abc}	1.24	***	NS	NS
ADL	42.65	43.02	49.57	52.60	53.64	1.48	**	NS	NS

Control: 0 g/kg diet sodium humate inclusion; 5HNa: 5 g/kg diet sodium humate inclusion; 7.5HNa: 7.5 g/kg diet sodium humate inclusion; 10HNa: 10 g/kg diet sodium humate inclusion and 12.5HNa: 12.5 g/kg diet sodium humate inclusion.
L: linear; Q: quadratic and C: cubic.
NDF: neutral detergent fibre; ADF: acid detergent fibre and ADL: acid detergent fibre.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
NS: non significant.

Table 5 Rate of mineral absorption (%) in West African Dwarf (WAD) goats fed concentrate diet containing incremental levels of sodium humate

Mineral	Treatment groups					SEM	Polynomial contrast		
	Control	5HNa	7.5HNa	10HNa	12.5HNa		L	Q	C
Zinc	60.26^{cd}	59.23^{d}	62.83^{bc}	66.39^{a}	64.70^{ab}	0.67	***	NS	***
Copper	51.45^{d}	61.81^{bc}	66.79^{a}	60.43^{c}	65.19^{ab}	1.28	***	***	***
Iron	88.55^{ab}	89.30^{a}	89.36^{a}	87.32^{b}	89.95^{a}	1.09	NS	NS	***
Manganese	63.38^{c}	64.32^{bc}	66.90^{ab}	68.23^{a}	68.57^{a}	2.40	***	NS	NS
Sodium	64.94^{d}	69.33^{c}	75.56^{a}	72.05^{b}	71.36^{bc}	3.70	***	***	NS

Control: 0 g/kg diet sodium humate inclusion; 5HNa: 5 g/kg diet sodium humate inclusion; 7.5HNa: 7.5 g/kg diet sodium humate inclusion; 10HNa: 10 g/kg diet sodium humate inclusion and 12.5HNa: 12.5 g/kg diet sodium humate inclusion.
L: linear; Q: quadratic and C: cubic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
NS: non significant.

Table 5 shows the rate (%) of mineral absorption of WAD goats on incremental levels of sodium humate. All the mineral absorption rates examined significantly (P<0.05) increased at sodium humate supplementation. Zinc (Zn) absorption rate were highest (P<0.05) in 10HNa and 12.5HNa groups, with least (P<0.05) absorption rates found in control and 7.5HNa groups. Copper (Cu) absorption rates were highest (P<0.05) in 7.5HNa and 12.5HNa groups and reduced (P<0.05) in 5HNa and 10HNa groups.

Iron (Fe) absorption rates were highest (P<0.05) in control, 5HNa, 7.5HNa and 12.5HNa groups and reduced (P<0.05) in the 10HNa group. Manganese (Mn) absorption rates were highest (P<0.05) in 7.5HNa, 10HNa and 12.5HNa groups with least (P<0.05) similar values of Mn absorption found in the control and 5HNa groups. Sodium (Na) absorption rate was highest (P<0.05) in 7.5HNa group with least (P<0.05) similar absorption rates observed in the control and 12.5HNa groups.

Tables 6a and 6b shows the oxidative stress biomarkers of semi-intensively raised WAD goats supplemented concentrate diet containing incremental levels of sodium humate. Malondialdehyde was significantly higher in the 7.5HNa, 10HNa and 12.5HNa groups during 30 days of supplementation but reduced (P<0.05) in the control and 5HNa groups. At 90 days, MDA had comparable highest (P<0.05) values in the control, 5HNa, 7.5HNa and 10HNa groups with least value of MDA in the 12.5HNa group. Superoxide dismutase was significantly higher in control and 10HNa groups at 30 days of feeding but reduced (P<0.05) in the other treatment groups. Glutathione peroxidase linearly increased (P<0.05) until 10HNa group, then decreased (P<0.05) in the 12.5HNa group during 30 days of supplementing sodium humate. However, not effect (P>0.05) on SOD and GSHPx was observed during 60 and 90 days supplementation of sodium humate. There was a linear increase (P<0.05) in albumin concentration at 90 days of sodium humate supplementation. Highest (P<0.05) similar albumin levels were observed in 5HNa, 7.5HNa, 10HNa and 12.5HNa groups with least (P<0.05) value of albumin found in control group. Nitric oxide at 90 days of supplementation reduced (P<0.05) with increased levels of sodium humate supplementation. Bilirubin had a reverse trend from NO as the highest (P<0.05) was found in control group at 90 days of feeding least (P<0.05) bilirubin was observed in 7.5HNa group at 90 days. Uric acid was only significantly (P<0.05) influenced at 90 days of sodium humate supplementation. It was observed that uric acid was highest (P<0.05) in the 10HNa group and this significantly decreased in the control and 5HNa groups.

The temperature-humidity index (THI) is a weather safety index that has been developed to measure heat stress in animals (Wiersma, 1990). The THI values recorded during the period of the experiment for morning and afternoon were above the threshold 27.5 reported by Popoola *et al.* (2014) to affect the growth of WAD goats. Under Egyptian environmental conditions, THI of 33.53 was said to be extremely stressful for goats (Teama, 2018). Given the circumstance, animals on this study would have been said to be under heat stress.

Improved weight in this current research may be due to reasons advanced about positive effect of humic substances in enhancing growth by way of increased metabolic activity of cell membranes. This action accelerates oxidative processes, which result in increased nutrient uptake and stimulation of certain vital functions (Islam *et al.* 2005) which are responsible for growth. In addition, the ability of humic acids to stabilize the intestinal flora of the animals, thereby improving utilization of nutrients may have been responsible for the improved growth (Humin Tech, 2004).

However, the increase in weight in the sodium humate fed groups did not have a definite pattern as weight of animals had both linear and quadratic increase. Result on enhanced growth in this current research agrees with findings of Cusack (2008) who reported improved growth performance in cattle when humates were used in their diets as natural antibiotics. In dairy operations, faster growth rates were observed by the use of humates (Livestock Resource US, 2003).

Similarly, Agazzi *et al.* (2007) indicated that average daily gain was increased in goats, which were orally supplemented with a humate solution at up to 30 mL/d at 4 to 8 weeks of age. On the contrary Chirase *et al.* (2000) reported that the addition of humates to feedlot diets did not significantly impact the average daily weight gain of cattle in a 56-day feeding trial. Degirmencioglu (2012) reported that diets with 0, 1.0 and 3.0 g HA kg^{-1} on live weight for goats showed no significant changes for live body weight. The difference in the work of Chirase *et al.* (2000) and Degirmencioglu (2012) with the current study could be due to differences in the management system, animal species and / or breed. Decrease in daily concentrate intake in the sodium humate supplemented groups may be due to increased lag time in passage of feed in the gut. Ikyume *et al.* (2020b) has predicted a decrease in dry matter intake (DMI) of WAD goats on diets containing 5, 7.5, 10 and 12.5 g HNa/kg diet using *in vitro* conditions. Result of DMI in the current study is consistent with the report of El-Zaiat *et al.* (2018) for goats on humic substance as organic additives. Covington *et al.* (1997) also reported that lambs fed 10.0 and 20.0 g HA/kg during a 63 day feeding period consumed less feed than 5.0 g HA/kg and control. Report by Brown *et al.* (2007) did not show differences in DMI for steers when a humate product was added to a high concentrate diet while that of McMurphy *et al.* (2011) reported greater DMI in Holstein steers in humates group compared to the control group.

The differences in DMI in this current research and that of other researchers may be due to specie difference and type of humic substance used in the experiments. The linear and quadratic response in the DMI is indicative that the action of sodium humate on DMI of WAD goats is not dose dependent. Feed conversion ratio also improved in the sodium humate groups. This increase in FCR may be due to the ability of humates to improve utilization of nutrient from the diet.

Apparent nutrient digestibility in this study was generally high. This implies all experimental diets in the study were adequately utilized. The quadratic and cubic relationship as observed for DM digestibility implies the effect of sodium humate on DM digestibility may be dose dependent.

Table 6a Stress biomarkers of West African Dwarf (WAD) goats supplemented concentrate diet containing incremental levels of sodium humate

Oxidative stress biomarkers	Duration	Treatment groups					SEM	Polynomial contrast		
		Control	5HNa	7.5HNa	10HNa	12.5HNa		L	Q	C
Malondialdehyde (U/L×10^{-09})	30 days	1.24^{bc}	1.16^{c}	1.58^{ab}	1.69^{a}	1.41^{abc}	0.07	*	NS	*
	60 days	1.65	1.78	2.10	1.74	2.07	0.10	NS	NS	NS
	90 days	3.83^{a}	2.69^{ab}	4.05^{a}	2.83^{ab}	1.91^{b}	0.28	*	NS	NS
Superoxide dimutase (U/L)	30 days	0.02^{a}	0.009^{b}	0.009^{b}	0.013^{ab}	0.008^{b}	0.001	*	NS	*
	60 days	0.0008	0.0007	0.0010	0.0011	0.0010	0.00008	NS	NS	NS
	90 days	0.007	0.007	0.012	0.008	0.008	0.0009	NS	NS	NS
Glutathione peroxidase (U/L)	30 days	5.33^{ab}	6.57^{a}	7.13^{a}	5.07^{ab}	2.53^{b}	0.48	**	**	NS
	60 days	7.63	6.10	7.77	5.10	6.23	0.51	NS	NS	NS
	90 days	5.93	2.63	6.90	7.00	5.40	0.55	NS	NS	NS
Albumin (g/L)	30 days	38.27	39.00	37.43	39.23	38.37	0.24	NS	NS	NS
	60 days	44.73	47.03	42.77	47.13	47.87	0.63	NS	NS	NS
	90 days	41.53^{b}	43.73^{ab}	44.50^{ab}	43.60^{ab}	46.07^{a}	0.55	*	NS	NS

Control: 0 g/kg diet sodium humate inclusion; 5HNa: 5 g/kg diet sodium humate inclusion; 7.5HNa: 7.5 g/kg diet sodium humate inclusion; 10HNa: 10 g/kg diet sodium humate inclusion and 12.5HNa: 12.5 g/kg diet sodium humate inclusion.
L: linear; Q: quadratic and C: cubic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
NS: non significant.

Table 6b Oxidative stress biomarkers of West African Dwarf (WAD) goats supplemented concentrate diet containing incremental levels of sodium humate

Oxidative stress biomarkers	Duration	Treatment groups					SEM	Polynomial contrast		
		Control	5HNa	7.5HNa	10HNa	12.5HNa		L	Q	C
Uric acid (mg/dL)	30 days	6.02	5.26	6.02	6.26	6.02	0.22	NS	NS	NS
	60 days	7.79	8.07	7.16	9.80	8.35	0.49	NS	NS	NS
	90 days	8.53^{b}	8.86^{b}	10.03^{ab}	11.26^{a}	9.37^{ab}	0.37	*	NS	*
Nitric oxide (µM)	30 days	6.10	5.93	5.47	4.73	6.80	0.29	NS	NS	NS
	60 days	4.73	6.17	5.83	5.37	4.67	0.35	NS	NS	NS
	90 days	6.03^{a}	4.70^{ab}	3.70^{b}	3.90^{b}	4.07^{b}	0.26	**	*	NS
Bilirubin (mg/dL)	30 days	0.60	0.53	0.57	0.43	0.57	0.02	NS	NS	NS
	60 days	0.60	0.53	0.43	0.60	0.60	0.04	NS	NS	NS
	90 days	0.70^{a}	0.63^{ab}	0.50^{b}	0.53^{ab}	0.67^{ab}	0.03	NS	*	NS

Control: 0 g/kg diet sodium humate inclusion; 5HNa: 5 g/kg diet sodium humate inclusion; 7.5HNa: 7.5 g/kg diet sodium humate inclusion; 10HNa: 10 g/kg diet sodium humate inclusion and 12.5HNa: 12.5 g/kg diet sodium humate inclusion.
L: linear; Q: quadratic and C: cubic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
NS: non significant.

Dry matter digestibility was above threshold of 60% recommended to be a highly digestible diet. Increased crude protein in the humate groups may be as a result of the trace minerals in humates which are components in numerous enzyme systems associated with carbohydrate and protein metabolism. Trace elements within humic substance may also act as cofactors in nitrogen digestibility, increasing the activity of enzymes for increased digestion and utilization of nutrients (Hayirli *et al*. 2005). However, this increase in crude protein digestibility may be dose dependent as it was decreased at levels beyond 7.5 g/kg diet inclusion of sodium humate. The decrease in the DM and protein digestibility coefficients at higher inclusion levels of sodium humate may be because of the ability of humate to construct different types of binding interactions that can affect nutrient resorption.

However, Pisarikova *et al.* (2009) reported that this ability to construct different types of binding interactions may be dose dependent and that, in terms of possible use of HNa as a nutritional additive, further experiments was needed to find the optimum dose of humate in feed mixtures. This is to say concentration above 7.5 g/kg diet appears to be too high if nutrient digestibility is to be enhanced. However, growth performance for all humate groups in this current study was observed to be similar amidst such decrease in DM and crude protein digestibility. Even though report of Terry *et al.* (2018) did not observe any significant difference in nutrient digestibility of a barley silage-based diet containing increasing concentrations of humic substances in beef heifers, higher supplementation levels (300 mg/kg BW humic substance) in the report of Terry *et al.* (2018) numerically increased protein digestibility.

The result of crude fibre digestibility showed similar trend with that of DM and CP digestibility, even though crude fibre digestibility increased at sodium humate supplementation, it was lowered at higher concentrations. Such effect could be as a result of sodium humate to improve the digestibility and nutrient metabolism by altering partitioning of nutrient in the feed (Parks *et al.* 1986; Stepchenko *et al*. 1991). The improved digestion coefficients of ash, ether extract, neutral detergent fibre and acid detergent fibre in this current study may be due to the augmented extension of enzymatic digestion and the lessening in passage rate of the intestinal content. On the contrary, reports of nutrient digestibility from ruminants did not indicate any differences in nutrient digestibility (Terry *et al.* 2018). The difference between this report and that of Terry *et al*. (2018) can be adjured to be specie of animal, environment and type and concentrations of humic substance used.

Grazing animals must absorb certain trace minerals to remain healthy and productive. It has been established that deficiencies of any of the minerals lead to a variety of negative effects (Soetan *et al.* 2010). Absorbability of micro nutrient in feed is the primary determinant of bioavailability and this is determined by the proportion of absorbed micro nutrient that is not excreted (retained micro nutrient). These retained micro nutrients are able to perform essential functions within the body system. The linear increase in absorption for all the trace minerals measured is indication that the levels of respective trace minerals in all the humate diets were not above the requirement of the animals. This is because animals will only absorb what is required (apart from copper) for metabolic activities and animals reduce absorptive efficiency when these minerals are provided in excess. The linear increase in mineral absorption in the humate group may be due to the structure of the sodium humate.

Humic substances display strong complexing abilities for a variety of different classes of compounds, thus, it is highly possible the humic and fulvic acids contained in sodium humates may bind to co-ingested compounds resulting in a direct interaction and altered absorption. The increase in absorption of Fe in this current study contradicts the insinuation of Ho *et al.* (2003) that, humic acid coupled with released iron can disturb the redox balance and prompt oxidative stress within a biological system. According to him, from the assessment of its redox properties, HA has been shown to be capable of reducing iron (III) to iron (II) in aqueous conditions over a broad range of pH values (from 4.0 to 9.0). In this report, in the presence of humic acid, Fe uptake was found to increase. The findings of this report agree with the report of Willis (2015), that, humic acid increased absorption of the poorly soluble ferrous iron (iron II) in the GIT. Willis (2015) has reported that intestinal absorption of co-ingested nutrient substances, like minerals and vitamins, in the diet or in supplement form, may also be affected by the presence of humic and fulvic acid. Mechanism of improved absorption by humic substance was explained in report of Willis (2015). First it could be that the binding of other minerals to the humic substance to form a mineral-humic substance complex has an effect on the uptake of the mineral or by altering the mineral physicochemical properties which is responsible for changes in the mineral solubility whereby a different transport mechanism in the intestinal membrane is utilized. This change may lead to a more or less suitable transport mechanism being used, leading to increases or decreases in absorption. Further research will be required to ascertain the mechanism by which sodium humate enhanced the absorption of trace minerals.

Malondialdehyde is a by-product of lipid peroxidation indicative of free radicals while NO, GSHPx and SOD are antioxidant enzymes that quench free radicals and are measured as biomarkers of oxidative stress. The linear increase in MDA concentration obtained in the 7.5HNa and 10HNa goats at 30 days of feeding may have ensued, seemingly, as a result of free-radical induced lipid peroxidation of the erythrocyte membranes. Such effects can be attributed to increased metabolic activity in the cell, which can result in increase in production of free radicals (Maurya *et al.* 2015). However, the action of sodium humate to increase MDA was limited to 30 days feeding as during 90 days, MDA decreased at 12.5HNa group. It is likely that the adjustment period to the diet might have been responsible for elevated levels of MDA during 30 days feeding of sodium humate. It may suggest that goats need to adapt to the supplement for four weeks before production response is detectable. Plasma SOD is considered the first defence against pro-oxidants (Machado *et al.* 2014). Plasma GSHPx activity is very important in protecting the organism against reactive oxygen specie (ROS) (Pilarczyk *et al.* 2012) and this makes them indicators of oxidative stress. The asymptomatic decrease observed in SOD and GSHPx at 30 days feeding may be due to increased metabolic activity arising from increase in the levels of MDA and this is consistent with finding of Ipek *et al.* (2008) that increase in levels of MDA was accompanied by a corresponding decrease in antioxidant capacity of the quail birds. In this current study, the decreased in SOD may have been due to adjustment period in the sodium humate supplemented groups, as such effect was not observed in the 60 and 90 days feeding. Albumin is considered an antioxidant pool because of its ability to play an important role against ROS (Kuciel-Lewandowska *et al.* 2020). The liver exclusively synthesizes albumin, and a reduction in liver function will mean lower levels of albumin. An increase in albumin concentration observed in this study at 90 days of feeding may due to

increased function in liver function. This is to say that feeding of sodium humates to the animals may have enhanced liver function because of improved immune response of the animals. Uric acid (UA) is a non-enzymatic low-molecular weight antioxidant (Kuciel-Lewandowska *et al*. 2020) and it is the final product of purine metabolism. The role of uric acid in conditions associated with oxidative stress is not entirely clear (Glantzounis *et al.* 2005), but report indicates that it is characterized by a slow, steady increase in plasma levels until long after completion of physical exercise (Zielinski *et al*. 2008). This action may be to prepare the animals for dealing with stress from physical exercise. The WAD goats in this study were reared on a semi-intensive system of management, which involves some physical exercise.

The increase in uric acid in this current study at higher levels of sodium humates used in this study may be indication that sodium humate improved antioxidant capacity of the animals. Apart from its action as radical scavenger, uric acid can also chelate metal ions, like iron and copper, converting them to poorly reactive forms unable to catalyse free-radical reactions (Pasalic *et al*. 2012). Nitric oxide and superoxide radical ($O_2^{-\bullet}$) rapidly react together to form a third free radical, peroxynitrite, thus serves as scavenger for $O_2^{-\bullet}$ (Lubos *et al.* 2009). This peroxynitrite is an important mediator of free radical toxicity with strong oxidizing properties towards biological molecules, including protein and non-protein sulfhydrates, deoxyribonucleic acids, and membrane phospholipids (Lubos *et al.* 2009). The balance between oxidative stress and anti-oxidative defence mechanisms may be impaired because of increased serum NO levels. In this report, during 90 days feeding, serum NO levels decreased in sodium humate groups, which is indication that the animals did not experience oxidative stress. There are interesting scientific facts about bilirubin acting as an endogenous scavenger of NO, giving him the role of antioxidant (Memic *et al*. 2012). Except for levels of bilirubin observed in 7.5HNa group, other sodium humate supplemented groups had similar bilirubin concentration with the control group. The decrease in bilirubin levels observed for 7.5HNa group may have been because of the NO scavenging activity of bilirubin, which is consistent with lower levels of NO in 7.5HNa group.

CONCLUSION

The findings of this study indicated that sodium humate supplementation up to 12 g/kg diet improved the growth, nutrient digestibility and antioxidant capacity of semi-intensively managed WAD goats.

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