

Blood profile and oxidative biomarkers in goats on cassava based diets containing varying levels of sodium humate

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Abstract

The detoxifying abilities of the humic acids and its antioxidant capacity have led to an increased interest in its use as feed additive in livestock production. This research assessed the physiological and antioxidant response of West African dwarf (WAD) goats to cassava peels based diets containing different levels of sodium humate. The experimental design was a completely randomized design, with four treatment diets containing sodium humate at 0, 5, 10 and 15 g/kg diet (Control, 5HNa, 10HNa and 15HNa). Twenty (20) WAD bucks were divided into four (4) groups of five (5) animals and assigned to the four experimental diets. Data was collected on haematological parameters, serum indices and oxidative stress biomarkers and statistically analyzed using the GLM of SPSS (version 23). Result revealed that sodium humate in cassava peels based diets of WAD goats did not affect ($p > 0.05$) the haematological parameters measured. Total protein increased ($p < 0.05$) in cassava peels based diet containing 5 g/kg diet (6.43 mg/dL) of sodium humate compared to the other groups (5.50, 5.75 and 5.99 mg/dL for control, 10HNa and 15HNa groups respectively). Also, sodium humate in cassava peels based diets reduce ($p < 0.05$) glucose level in the blood compared to the control. Serum aluminum increased ($p < 0.05$) in 10HNa group ($0.33 \mu\text{gL}^{-1}$) compared to the 5HNa group ($0.30 \mu\text{gL}^{-1}$) but not in the control and 15HNa groups ($0.31 \mu\text{gL}^{-1}$, respectively). Albumin concentration increased ($p < 0.05$) in all sodium humate groups (5.23, 5.50 and 5.48 mg/dL for %HNa, 10HNa, and 15HNa, respectively) compared to the control (3.50 mg/dL). Uric acid increased ($p < 0.05$) with the increasing amount of sodium humate in the cassava peels based diets. In conclusion, to address the concerns of possible cyanide poisoning and mycotoxins in the mixed diets containing cassava peels, up to 15 g/kg diet of sodium humate could be used as additive to improve the physiological and antioxidant capacity of the animals.

Key words: Cassava peels, WAD goats, antioxidant, sodium humate, Blood profile

Introduction

Cassava peels have continued to gain popularity as feed resources for ruminant production. This is more so with the increased production and processing of cassava with the peels left as

by-products of cassava processing. The peels as unconventional feedstuff, serves as alternative to maize, thereby reducing the high cost arising from competition for maize between livestock and humans. However, the consumption and utilization of cassava peels by ruminants is

constrained due to the presence of cyanide. Ruminants unlike monogastric animals are more susceptible to cyanide poisoning because of enzymes in the rumen microflora which increases hydrolysis of cyanogenic glycosides (Nicholson, 2012). It could also be that the higher rumen pH in ruminants compared to monogastric animal favours a conversion of more hydrocyanic acid (Radostits et al., 2007; Pickrell and Oehme, 2013). It is therefore, imperative to adopt strategies that will reduce the incidences of cyanide poisoning in ruminants in order to increase the utilization of cassava peels as feedstuff for ruminants.

The concentration of hydrocyanic acid (HCN) can be considerably reduced or even eliminated through processing and one of such processing method is drying. At the traditional level, cassava peels are sun dried on the ground, with proper drying achieved in 1-3 days during the dry seasons while this may take up to eight days or more during the rainy season (Amole et al., 2019). The traditional sun drying is slow and encourages the growth of mould and several other microorganisms that may expose livestock to aflatoxicosis and/or mycotoxic infection after feeding on them. This is likely to lead to oxidative stress in the animals and consequently a reduced performance. These challenges (cyanide poisoning and mycotoxins in dried peels) have made the livestock feed industry and other direct users of cassava peel to be cautious of its usage following concerns about the safety and hygiene of the product.

Given that the occurrence of mycotoxin has remained a global significant challenge, with attendant rising animal and human health hazards and huge financial losses in the feed and food production industries (EFSA, 2011; Pinotti et al., 2016), I propose that the supplementation of natural complexes of biologically active substances with antioxidant, adsorbent and antibiotic potentials can make dried cassava peels safe for livestock feed will alleviate the concerns (cyanide poisoning and mycotoxins) about its usage in the feed industry. The utilization of feed additives that decrease animal exposure to mycotoxins can be in the form of a substance blended

in to the feed (in form of mineral clay, micro-organism, yeast cell wall) to absorb or detoxify mycotoxins in the digestive tract of animals (biological detoxification) (Boudergue et al., 2009). It is important that biological agents are used as feed additives should majorly degrade mycotoxins into non-toxic metabolites, and must be safe and stable in the gastrointestinal tract of animals. One of such feed additives is the use of alkaline salts of natural humic acids and one of which is sodium humate.

Humic substances (HS) are complex mixtures of high-molecular organic compounds of natural origin. Humic acids inhibit bacterial and fungal growth, thus indirectly decreasing levels of mycotoxins in feed (Riede et al., 2007). Some humic substances and their salts have been described to directly interact with mycotoxins by their mycotoxin binding capacity (Sabater-Vilar et al., 2007; Ye et al., 2009). This action is likely to improve the health and antioxidant capacity of the animals thereby by ensuring their welfare. This research therefore, was designed to assess the blood profile and antioxidant capacity of West African dwarf goats on cassava peels based diet with varying levels of sodium humate.

Materials and Methods

Experimental site: The feeding trial was conducted at the Animal Science Teaching and research far of Joseph Sarwuan Tarka University (formerly University of Agriculture) Makurdi. The area temperature range of 24.20 ± 1.40 °C and 36.33 ± 3.70 °C (TAC, 2009).

Experimental design and animal management: The feeding trial lasted for eleven (11) weeks after six (6) weeks of allowing the animals to acclimatize to the new environment. A total of twenty (20) West African dwarf (WAD) bucks weighing between 5.54–11.39 kg were purchased from local farmers in wannune and Ikpayongo Markets of Benue State, Nigeria. During the period of acclimatization, the animals were given prophylactic treatment to ensure uniform health condition before the commencement of the ex-

periment. They were administered PenStrep (1 ml/10 kg) intramuscularly against possible bacterial diseases. They were also treated for ecto- and endoparasite using Ivermectin (0.2 ml/10 kg) subcutaneously. The bucks were housed individually in separate pens measuring 1 x 1.5 m each. The pens were made of slated floor and a corrugated zinc roof constructed to ensure free flow of air. The bucks were offered experimental concentrate diet (Table 1) in the morning (7.00 am) and forage (*Panicum maximum*) in the afternoon (12.00 noon) and fresh clean water was provided ad-libitum. The bucks were kept under hygienic and ethical conditions, and were confined throughout the experimental period.

The experimental design was a completely randomized design (CRD). Four experimental diets were formulated as follows: Control (0 g/kg diet of sodium humate), 5HNa (5 g/kg diet of sodium humate), 10HNa (10 g/kg diet of sodium

humate) and 15HNa (15 g/kg diet of sodium humate)

Data collect: By the 77th day of the feeding trial, 7 ml of blood samples were collected from all twenty (20) animals to determine their haematological parameters (packed cell volume, haemoglobin white blood cells, red blood cells and phagocytes), serum indices (total protein, globulin, glucose, total cholesterol, triglyceride, HDL and LDL), serum minerals (Na, Ca, Mn, Cu, Al and Zn) and serum oxidative stress biomarkers (albumin, uric acid, SOD, glutathione peroxidase, MDA). Blood samples for haematological indices were emptied into sample bottles containing ethylene tetra acetic acid (EDTA), to prevent blood clotting while samples for serum analysis were collected into bottles without EDTA to allow for clotting to harvest the serum. Haematological parameters such as RBC, WBC, and lymphocyte counts were evaluated utilizing an automatic blood analyzer (ADVIA 120, Bayer, USA). Packed cell volume (PCV) and hemoglobin (Hb) were determined using the microhaematocrit method and cyanmethemoglobin method, respectively. The serum total protein concentration was determined using Biuret reagent as described by King and Wooton (1965). Plasma glucose concentration was determined by the enzymatic colorimetric method using a kit (Spinreact, S.A., Spain). The amounts of total Cholesterol, triglyceride, HDL, LDL was spectrophotometrically determined on an Olympus Au 400 system autoanalyzer. The procedures described in Afele et al. (2020) were adopted for the determination of serum minerals and oxidative stress biomarkers.

Table 1. Gross composition and chemical analysis of experimental diets

| Ingredient | Treatments | | | |
|-------------------------|------------|-------|-------|-------|
| | Control | 5HNa | 10HNa | 15HNa |
| Maize offal | 28 | 28 | 28 | 28 |
| Cassava peel | 32 | 32 | 32 | 32 |
| Soybean meal | 20 | 20 | 20 | 20 |
| Rice offal | 12 | 12 | 12 | 12 |
| Bone meal | 6 | 6 | 6 | 6 |
| Vitamin premix | 1 | 1 | 1 | 1 |
| Salt | 1 | 1 | 1 | 1 |
| Total | 100 | 100 | 100 | 100 |
| Sodium humate (g) | 0 | 500 | 1000 | 1500 |
| Determined analysis | | | | |
| Dry matter | 86.32 | 85.93 | 86.46 | 86.70 |
| Crude protein | 12.86 | 12.92 | 12.94 | 13.01 |
| Ash | 5.43 | 6.32 | 6.43 | 6.57 |
| Ether extract | 4.13 | 4.21 | 4.06 | 4.16 |
| Neutral detergent fibre | 57.32 | 56.10 | 54.04 | 55.46 |
| Acid detergent fibre | 18.43 | 19.00 | 23.00 | 20.00 |

Control – 0 g/kg diet of sodium humate inclusion

5HNa – 5 g/kg diet of sodium humate inclusion

10HNa – 10 g/kg diet of sodium humate inclusion

15HNa – 15 g/kg diet of sodium humate inclusion

Results

Table 2 represent the haematological and phagocytes of West African dwarf goats on a cassava peels based diet containing different levels of sodium humate. All haematological parameters measured were not affected ($p > 0.05$) by addition of sodium humate in the diets.

The serum indices of West African dwarf goats as on cassava peel based diet as affected

Table 2. Haematological parameters of West African dwarf goats fed cassava-based diets containing different levels of sodium humate

| Parameter | Treatment Diets | | | | |
|--|-----------------|-------|-------|-------|------|
| | Control | 5HNa | 10HNa | 15HNa | SEM |
| Packed cell volume (%) | 29.00 | 28.75 | 27.75 | 28.75 | 0.63 |
| Red blood cells ($\times 10^{12/l}$) | 15.60 | 14.80 | 13.73 | 15.65 | 0.67 |
| White blood cells ($\times 10^9/l$) | 9.70 | 6.40 | 6.90 | 8.53 | 0.67 |
| Haemoglobin (g/dl) | 9.68 | 9.68 | 8.75 | 9.50 | 0.23 |
| Mean corpuscular volume (fl) | 17.93 | 19.88 | 20.30 | 19.60 | 0.95 |
| Mean corpuscular haemoglobin (pg) | 46.28 | 66.10 | 67.78 | 64.65 | 4.00 |
| MCHC (g/dl) | 33.33 | 33.30 | 33.35 | 33.35 | 0.03 |
| Lymphocyte (%) | 65.75 | 63.25 | 66.75 | 67.50 | 1.62 |
| Neutrophil (%) | 27.75 | 31.00 | 30.00 | 27.00 | 1.48 |
| Eosinophil (%) | 2.00 | 1.75 | 0.75 | 1.50 | 0.30 |
| Basophil (%) | 0.25 | 0.00 | 0.00 | 0.25 | 0.10 |
| Monocyte (%) | 3.00 | 3.50 | 3.00 | 3.50 | 0.32 |

Control – 0 g/kg diet of sodium humate inclusion

5HNa – 5 g/kg diet of sodium humate inclusion

10HNa – 10 g/kg diet of sodium humate inclusion

15HNa – 15 g/kg diet of sodium humate inclusion

by different level of sodium humate in the diets is as shown in Table 3. Total protein increased ($p < 0.05$) in 5HNa group (6.43 mg/dL) (5 g/kg diet of sodium humate) compared to the control group (5.50 mg/dL) (no sodium humate). However, there were comparable concentrations of total protein in control, 10HNa and 15HNa groups (5.50, 5.75 and 5.99 mg/dL, respectively). Inclusion of sodium humate in cassava peel based diet reduced ($p < 0.05$) glucose concentration of WAD goats. Comparable values were however observed for all sodium humate groups (23.43, 25.70 and 28.33 mg/dL for 5HNa, 10HNa and 15HNa, respectively). All other serum indices measured were not affected ($p > 0.05$) by the inclusion of sodium humate in the cassava peel based diet.

The result of the blood mineral indices of West African dwarf (WAD) goats fed cassava peels based diet containing different levels of sodium humate is presented in Table 4. All blood mineral indices measured except aluminum were not affected ($p > 0.05$) by the inclusion of different levels of sodium humate in the diets. Aluminum increased ($p < 0.05$) in T3 ($0.33 \mu\text{g/L}^{-1}$) compared

to T2 ($0.30 \mu\text{g/L}^{-1}$). The concentration of aluminum was same in both T1 and T4 ($0.31 \mu\text{g/L}^{-1}$).

Table 5 represents oxidative stress biomarkers of West African dwarf goats fed cassava-based diet containing different levels of sodium humate. Albumin concentration increased ($p < 0.05$) in all sodium humate groups (5.23, 5.50 and 5.48 mg/dL for 5HNa, 10HNa and 15HNa, respectively) compared to control group (3.50 mg/dL). On the other hand, uric acid increased ($p < 0.05$) with the increasing amount of sodium humate in the cassava peel based diet. The control group had a concentration of 6.85 mg/dL while values of 10.68, 11.50 and 12.31 mg/dL were found in 5HNa, 10HNa and 15HNa, respectively. Other biomarkers such as MDA, SOD and glutathione peroxidase were not influenced ($p > 0.05$) by sodium humate in the diets.

Discussion

The non-significant difference in haematological parameters is consistent with reports of Ikyume (2021) for semi-intensively managed

Table 3. Serum indices of West African dwarf goats fed cassava-based diet containing different levels of sodium humate

| Parameter | Treatment Diets | | | | SEM |
|---------------------------|--------------------|--------------------|--------------------|--------------------|------|
| | Control | 5HNa | 10HNa | 15HNa | |
| Total protein (mg/dL) | 5.50 ^b | 6.43 ^a | 5.75 ^{ab} | 5.99 ^{ab} | 0.15 |
| Glucose (mg/dL) | 46.48 ^a | 23.43 ^b | 25.70 ^b | 28.33 ^b | 2.67 |
| Total cholesterol (mg/dL) | 126.18 | 134.93 | 145.73 | 128.95 | 4.95 |
| Triglyceride (mg/dL) | 180.98 | 178.05 | 185.70 | 173.50 | 3.36 |
| HDL (mg/dL) | 21.45 | 23.30 | 22.70 | 23.33 | 0.61 |
| LDL (mg/dL) | 64.85 | 76.88 | 73.30 | 70.88 | 5.59 |

^{a,b} Means with different superscript along the row are significant ($p < 0.05$)

Control – 0 g/kg diet of sodium humate inclusion

5HNa – 5 g/kg diet of sodium humate inclusion

10HNa – 10 g/kg diet of sodium humate inclusion

15HNa – 15 g/kg diet of sodium humate inclusion

SEM – Standard error of mean

Table 4. Blood mineral concentration of West African dwarf goats fed cassava-based diet containing different levels of sodium humate

| Parameter | Treatment Diets | | | | SEM |
|-----------------------------------|--------------------|-------------------|-------------------|--------------------|--------|
| | Control | 5HNa | 10HNa | 15HNa | |
| Calcium (μgL^{-1}) | 0.41 | 0.41 | 0.44 | 0.42 | 0.006 |
| Manganese (μgL^{-1}) | 0.64 | 0.62 | 0.65 | 0.62 | 0.005 |
| Aluminium (μgL^{-1}) | 0.31 ^{ab} | 0.30 ^b | 0.33 ^a | 0.31 ^{ab} | 0.004 |
| Copper (μgL^{-1}) | 0.67 | 0.68 | 0.71 | 0.71 | 0.05 |
| Sodium (μgL^{-1}) | 0.27 | 0.26 | 0.29 | 0.28 | 0.007 |
| Zinc (μgL^{-1}) | 0.06 | 0.06 | 0.06 | 0.06 | 0.0005 |

^{a,b} Means with different superscript along the row are significant ($p < 0.05$)

Control – 0 g/kg diet of sodium humate inclusion

5HNa – 5 g/kg diet of sodium humate inclusion

10HNa – 10 g/kg diet of sodium humate inclusion

15HNa – 15 g/kg diet of sodium humate inclusion

SEM – Standard error of mean

Table 5. Oxidative stress biomarkers of West African dwarf goats fed cassava-based diet containing different levels of sodium humate

| Parameter | Treatment Diets | | | | SEM |
|---|-------------------|--------------------|---------------------|--------------------|------|
| | Control | 5HNa | 10HNa | 15HNa | |
| Albumin (mg/dL) | 3.50 ^b | 5.23 ^a | 5.50 ^a | 5.48 ^a | 0.23 |
| Uric acid (mg/dL) | 6.85 ^c | 10.68 ^b | 11.50 ^{ab} | 12.31 ^a | 0.56 |
| Malondialdehyde (unit/mg protein) | 0.63 | 1.03 | 0.57 | 0.65 | 0.82 |
| Superoxide dismutase (U/L) | 127.45 | 125.58 | 141.65 | 122.93 | 3.91 |
| Glutathione peroxidase ($\mu\text{g/mg protein}$) | 101.65 | 93.18 | 115.23 | 93.48 | 6.51 |

^{a,b} Means with different superscript along the row are significant ($p < 0.05$)

Control – 0 g/kg diet of sodium humate inclusion

5HNa – 5 g/kg diet of sodium humate inclusion

10HNa – 10 g/kg diet of sodium humate inclusion

15HNa – 15 g/kg diet of sodium humate inclusion

SEM – Standard error of mean

West African dwarf goats supplemented concentrate diets containing sodium humate. However, the lack of differences in phagocytes contrast that report. This may be due to the different management systems adopted in the different research. Animals on semi-intensive management system are exposed to other external factors including physical stress, warm infestation that can influence the concentration of phagocytes. It should be noted that the values for haematological parameters and phagocytes are closely related to those reported for healthy WAD goats raised under tropical environment (Makurdi) as this experiment (Ikyume et al., 2018).

Increased total protein in the 5HNa group and not 10HNa and 15HNa compared to the control group in this study implies 5 g/kg sodium humate in cassava based diet gave optimum protein utilization from the diet. Sodium humate has been reported to increase total in semi-intensively managed WAD goats (Ikyume, 2021) and this activity is because of the ability of humic substances to increase activities of enzymes associated with glycolysis and nitrogen assimilation (Nunes et al., 2019). Cassava peels contain a high level of structurally indigestible carbohydrates (cellulose, hemicellulose, pectin, and lignin) and high anti-nutrients (hydrogen cyanide, tannin, and phytate) coupled with low protein content (Oloruntola et al., 2018). The use of sodium humate in a cassava peel based diet could therefore improve the utilization of the form of nitrogen in the cassava peel. It should be noted that levels beyond 5 g/kg diet of sodium humate tended to reduce the possible assimilation of nitrogen. This is to say that the action of sodium humate to improve assimilation of structurally indigestible molecules may be possible only under lower levels of inclusion.

The interaction of a living cell with humic acids binds heavy metal ions into stable chelate complexes, intercepts molecules of pesticides and other organic xenobiotics, and also binds free radicals formed in the plasma membrane as a result of lipid peroxidation (Simakova et al., 2021). Such interaction results in the release of energy which, instead of being spent on compensating for the adverse effects of the external environment, is used by the cell itself for growth

and reproduction, which ultimately leads to an increase in its competitiveness (Biryukov, 2006; Buzlama, 2006). The reduced glucose in the sodium humate groups may be as a result of the action of sodium humate to intercept toxic molecule that could be present in cassava peels. This interaction between living cells and humic acids in the sodium humate may have resulted in the increased uptake of energy by the cells for growth. Such increase in energy uptake could lead to less glucose available in the blood. Ikyume (2021) observed an increase in glucose in WAD goats managed semi-intensively and supplemented with sodium humate at 60 days. However, the report of Degirmencioglu (2012) did not observe any difference in glucose for goats of humic substances. The contrasting results of other research works and this present study could be the duration of feeding and the possible non presence of possible toxins and cyanide in the other diets.

Studies have also demonstrated that the retention of mineral elements on an immune response is influenced by the nutrition plane and environmental management (Silva et al., 2013; Ji et al., 2014; Zhang et al., 2015). Increased AI in 10HNa group compared to 5HNa group observed in this study may have ensured seemingly due to individual animal differences, and not the increasing amount of sodium humate in the diet. This is because comparable results of AI concentration were observed in the control, 5HNa and 15HNa groups.

Albumin has been used as a measure of antioxidant status of an animal. This is because it is directly synthesized in the liver and its amount in the blood is correlated with the function of the liver. Increased level of albumin in sodium humate groups implies the inclusion supported maximum function of the liver of WAD goats on cassava peels based diets. Humic acids slip between the villi of the intestinal epithelium thereby creating a protective film from the finest particles of humic acid. This then, protects the tissues of the epithelium and lymph glands and slows the absorption of toxic materials, increasing their excretion with feces (Simakova et al., 2021) and may reduce the activity of the liver from handling toxic materials. Such increase in serum albumin in WAD goats on a sodium hu-

mate diet is consistent with report of Ikyume et al. (2021) for semi-intensively managed WAD goats on sodium humate diets. Uric acid as a non-enzymatic molecule is also involved in antioxidant activity in animals. Uric acid can chelate metal ions like copper and convert them to poor forms that are unable to speed free radical reactions (Pasalic et al., 2012). Increased amount of uric acid in all sodium humate groups support the antioxidant properties of sodium humate. With an increased antioxidant capacity, animals are able to handle challenges associated with possible toxic substances in the diet. Increased uric acid in the blood serum is consistent with report of Ikyume et al. (2021).

Conclusion

To address the concerns of possible cyanide poisoning in cassava peels and also the presence of mycotoxins arising from processing, the use environmentally friendly technologies for the production of livestock products, including natural complexes of biologically active substances with the properties of antioxidants, adsorbents and antibiotics can be adopted for use. The inclusion of alkaline salts of natural humic acids (sodium humate) in feed as additives is advocated. Sodium humate inclusion at 5 g/kg diet of feed supported optimum physiological responses in West African dwarf goats on cassava peels based diet for improved performance.

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