Immune and Oxidative Stress Indices of Yankassa Rams Fed Diets Containing Urea-Molasses Treated Cassava Peel Ensiled with Caged-Layer Droppings

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Abstract

This study evaluated the immune and oxidative stress responses of sheep fed diets containing urea-molasses treated cassava peels ensiled with caged layer droppings. The trial was conducted at Morugo Farms and Co-farms Greenaid Revolution Teaching and Research Farm, Abuja. A total of twenty-one clinically healthy Yankassa sheep, aged 6 to 7 months, were randomly assigned to dietary treatments in a completely randomized design and monitored over a 63-day feeding period. Daily feed intake was recorded, and physiological parameters were assessed to determine treatment effects. Key indicators of immune function—namely immunoglobulins G, A, and M—and metabolic hormones such as triiodothyronine (T3) showed no significant differences ($P > 0.05$) across all treatment groups compared to the control. Similarly, antioxidant markers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and total antioxidant capacity (TAC), remained unaffected by dietary treatments. Additionally, biomarkers of oxidative stress, such as malondialdehyde (MDA) and cortisol, were not significantly altered ($P > 0.05$), even at 25% and 50% replacement levels of the ensiled cassava peels diet. In conclusion, replacing conventional feed components with urea-molasses treated cassava peels ensiled with caged layer droppings up to 50% had no detrimental effects on immune status or oxidative stress responses in sheep. These findings support the use of this alternative feed resource within recommended nutritional standards for small ruminants, offering a sustainable and cost-effective option for improving feed availability without compromising animal health or performance.

Keywords; *Sheep nutrition, urea-molasses treated cassava peel silage, caged layer manure, physiological response, immune function, oxidative stress biomarkers, alternative feed resources*

Introduction

In many developing countries, including Nigeria, the average daily intake of animal protein (approximately 4.5 g/person/day) falls drastically short of the 35 g per day recommended by the Food and Agriculture Organization (FAO, 2001; Olafadehan et al., 2023a; Anaso et al., 2024b). This significant protein deficit underscores the urgent need to develop alternative, cost-effective strategies to enhance animal productivity, reduce production costs, and ensure the availability of high-quality animal-derived protein for consumers (Anaso et al., 2021b; Anaso et al., 2024b; Anaso et al., 2025a; Anaso et al., 2025c).

According to Anaso (2025b), optimized nutrition plays a pivotal role in improving the physiological and productive performance of livestock. While several studies have explored the impact of diverse feed resources on reproductive performance and physiological parameters in ruminants—particularly cattle—there remains a relative paucity of research focusing on small ruminants such as goats and sheep. Moreover, the specific mechanisms by which diet modulates reproductive and physiological responses in ruminants are still not fully understood (Anaso et al., 2024a; Anaso & Olafadehan, 2025).

Addressing the animal protein gap requires holistic approaches that enhance the nutritional profile, physiological adaptability, and reproductive efficiency of indigenous breeds, thereby increasing overall productivity and resilience in smallholder systems (Anaso et al., 2023; Anaso et al., 2025bc; Anaso et al., 2025b).

Although cassava peels and similar agro-industrial by-products are often regarded as low in crude protein and known to contain anti-nutritional factors such as cyanogenic glycosides, they have nonetheless been incorporated into ruminant diets (Anaso et al., 2021a; Anaso et al., 2021b; Olafadehan et al., 2023b). To mitigate the toxicity and preserve nutrient quality, various processing methods—including sun drying, soaking, retting, and particularly ensiling—have been employed to detoxify cyanide and reduce phytate content (Oboh, 2006; Olafadehan, 2011; Anaso et al., 2025d).

Furthermore, the nutritional quality of fibrous feedstuffs such as cassava peels can be enhanced through the inclusion of poultry manure and molasses. Ensiling cassava peels with caged-layer droppings and molasses not only detoxifies harmful compounds but also improves palatability and nutritive value (Anaso, 2025a).

This study therefore aimed to assess the immune and oxidative stress responses of Yankassa rams fed diets in which corn bran was partially replaced with cassava peels ensiled with molasses and caged-layer droppings. The goal was to explore the potential of this unconventional feed resource to sustainably enhance small ruminant productivity.

Materials and Method

Study area

The study was carried out at Morugo Farms in partnership with Co-farms Greenaid Revolution Teaching and Research Farm, Abuja. The research site is geographically located between latitudes 08°51' and 09°37' North and longitudes 07°20' and 07°51' East, within the southern Guinea savannah agro-ecological zone of Nigeria. The area is characterized by a bimodal climate, receiving an annual rainfall ranging from 1,145 mm to 1,631 mm. Ambient temperatures during the rainy season typically range from 25.8°C to 30.2°C, while the dry season is markedly hotter, with temperatures rising between 36°C and 42°C. According to Anaso (2025) and Anaso and Alhassan (2025), relative humidity in the region averages around 60% during the wet season but declines to approximately 30% in the dry season.

Source of feed ingredients and experimental animals

The cassava peels which were used for the experimental diet were obtained from selected farms during peeling of cassava in the post harvesting operation. Caged layer droppings were gotten from poultry farms within the environment, while molasses and urea were gotten from Gwagwalada Central market. The experimental rams were purchased from a nearby reputable animal farm Abuja Nigeria.

Experimental animals, management and diets

Twenty-one clinically healthy rams of Yankassa breed about 6 to 7 months with average initial body weight (BW) of 18.30 ± 0.46 kg were used for the experiment. The animals were ascertained to be healthy by a veterinary consultant, having vital parameters within normal ranges and showing no sign of disease. One week prior to the arrival of the animals, the surroundings and pens were thoroughly cleaned and disinfected with a strong antiseptic (morigad). On arrival, the rams were administered prophylactic treatment which included the intramuscular administration of oxytetracycline L.A. (Long acting) an antibiotic (1ml/10kg BW) against bacterial diseases. Ivomectin (0.5ml/25BW) was subcutaneously administered to the animals to protect them against endo-parasites inside the body cavity and ecto-parasites on the skin. Vitalyte was orally administered as anti-stress for a week in drinking water, before the animals were individually penned. Prior to isolation in their pen, they were allowed to adapt to their environment and the experimental diet for the period of one week before the commencement of experiment. The cages were cleaned thoroughly once a week.

Rams were weighed for their initial weight before starting feeding trials and they were randomized into three groups of similar body weight in completely randomized design (CRD). Experimental diets were fed to the animals and water given *ad libitum* through experimental period of 63 days.

100kg of dried cassava peel was collected, washed and allowed to wilt for 2 hours and mixed with 20kg of cage layers droppings and 10kg of molasses. It was allowed to for 30 days, then sundried for 3 days and grinded before taking to the laboratory for other analysis. The total amount of feeds offered daily and representative samples were on weekly basis and pooled together after the experiment. The quantity of feed provided and residue of each day was weighed to determine the feed intake of individual animal. The initial body weights of the goats were taken at commencement of the experiment and subsequently at 7 days interval in the morning before feeding.

Experimental diet

Three experimental diets were formulated with the inclusion of the three different types of supplements on cassava peel which include the cassava peel + molasses + cage layer droppings. T1 indicates 0% treated cassava peel replacement, T2 indicates 25% replacement of maize with treated cassava peel, and, T3 indicates the replacement of maize with 50% of treated cassava peel.

Table 1: Experimental diet with different inclusion level of treated cassava peel with urea-molasses ensiled with cage layer droppings on West Africa dwarf goat.

Ingredients (kg)	Treatment 1 0%replacmet of cassava peel	Treatment 2 25% replacement of cassava peel	Treatment 3 50% replacement of cassava peel
Maize	28	21	14
Treated cassava peel	0	7	14
GNC	18	18	18
Corn bran	21	21	21
TSH	28	28	28
Limestone	2	2	2
Salt	1	1	1
Urea	1	1	1
Vitamin premix	1	1	1
Cp	15.90	15.95	15.99
Total	100	100	100

Collection of parameters

Immune function was assessed by quantifying immunoglobulin classes (IgA, IgG, and IgM), also using Stanbio-specific kits per Elghalid et al. (2020).

Oxidative stress markers evaluated included triiodothyronine (T3), total antioxidant capacity (TAC), and the enzymatic activities of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and cortisol. These were likewise analyzed using the aforementioned commercial kits and standardized methodologies.

Statistical analyses

Data on body temperature regulation, serum biochemical markers, immune function, oxidative stress parameters, and semen characteristics were analyzed using analysis of variance (ANOVA) under a completely randomized design, employing SPSS version 23.0. To determine statistically significant differences among treatment means, Duncan's multiple range test was applied, with significance set at $P \leq 0.05$.

Results and Discussions

Immune and oxidative stress responses of Yankassa rams fed diets containing urea-molasses treated cassava peel ensiled with caged-layer droppings

Table 2 presents the immunological and oxidative stress responses of Yankassa rams fed diets incorporating urea-molasses treated cassava peels ensiled with caged-layer droppings. Across all treatment groups—including 25% (T2) and 50% (T3) dietary replacements—there were no significant differences ($P > 0.05$) observed in key immune markers, such as immunoglobulins G, A, and M, or in the metabolic hormone triiodothyronine (T3), when compared to the control group. Similarly, oxidative stress biomarkers, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and total antioxidant capacity (TAC), were unaffected by the dietary treatments. Moreover, stress indicators such as malondialdehyde (MDA) and cortisol concentrations also remained statistically unchanged ($P > 0.05$) across the different dietary inclusions.

Immunoglobulins—namely IgG, IgA, and IgM—are essential components of the adaptive immune system that contribute to host defense by neutralizing pathogens, blocking toxins, and preventing microbial adherence to mucosal surfaces (Woof & Kerr, 2005; Anaso et al., 2025a). The lack of significant variation in immunoglobulin levels across treatment groups suggests that the inclusion of the ensiled cassava peels did not compromise immune functionality.

Similarly, triiodothyronine (T3), a key thyroid hormone that governs metabolic activity, thermoregulation, and overall physiological stability (Bowen, 2010), was not altered by the test diets, indicating that basal metabolic functions remained intact.

Malondialdehyde (MDA), a marker for lipid peroxidation and oxidative membrane damage, remained stable across treatments, reflecting no apparent increase in oxidative stress levels. The unaltered MDA levels imply that the diets did not elicit excessive production of free radicals or compromise lipid integrity (Anaso et al., 2025a).

Cortisol, a glucocorticoid hormone released in response to physiological stress or hypoglycemia, also remained unchanged, highlighting the absence of stress-inducing effects from the experimental diets and suggesting good adaptation by the animals to the feed composition (Howhn & Marieb, 2010; Anaso, 2023a, 2023b).

Superoxide dismutase (SOD), a critical antioxidant enzyme that catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, was not significantly affected, suggesting that no additional oxidative challenge was imposed by the diets. The stability of SOD activity further implies adequate micronutrient availability required for optimal enzymatic function (Ashour et al., 2014).

The activities of glutathione-dependent enzymes—glutathione peroxidase (GPx) and glutathione reductase (GR)—also remained consistent among treatment groups. These enzymes play indispensable roles in mitigating oxidative damage and maintaining redox balance by neutralizing reactive oxygen species (ROS) and preserving the functional integrity of biomolecules (Anaso et al., 2025a). Their unchanged activity supports the conclusion that the test diets did not disrupt intracellular antioxidant defense mechanisms.

Additionally, enzymes such as CAT, GPx, and GR, which collectively serve to neutralize hydrogen peroxide and other peroxides, showed no significant differences among groups. These enzymes are vital for protecting cellular structures by catalyzing the breakdown of hydrogen peroxide into water and oxygen, thereby mitigating potential oxidative injury (Chelikani et al., 2004; Deponte, 2013).

In summary, the incorporation of urea-molasses treated cassava peels ensiled with caged-layer droppings at up to 50% dietary replacement did not adversely affect immunological function, oxidative stress parameters, or systemic physiological stability in Yankassa rams. These findings reinforce the potential of this alternative feed resource as a safe and nutritionally viable component in small ruminant diets.

Table 2. Immune and oxidative stress responses of experiment animals

Parameter	T1	T2	T3	SEM
Immunoglobulin G (mg/100ml)	4001.67	3996.67	3965.33	23.29
Immunoglobulin A (mg/ml)	0.41	0.38	0.37	0.00
Immunoglobulin M (mg/ml)	4.07	3.96	3.92	0.06
Triiodothyroxine (nmol/L)	2.10	1.99	1.96	0.05
Melanodialdehyde (nmol/L)	0.50	0.52	0.56	0.02

Superoxide dismutase (nmol/mL)	1001.67	997.00	994.00	3.20
Catalase (U/gHb)	2001.33	1999.00	1996.00	2.23
Glutathione peroxidase (U/gHb)	203.00	198.33	195.33	2.96
Cortisol (ng/ml)	9.99	10.30	10.37	0.42
Total antioxidant capacity (nmol)	1.28	1.13	0.99	0.27
Glutathione reductase (IU/10^{11}RBC)	1.81	1.78	1.75	0.03

Means in the same row are statistically different at ($P<0.05$), T1 indicates 0% treated cassava peel replacement, T2 indicates 25% replacement of maize with treated cassava peel, and, T3 indicates the replacement of maize with 50% of treated cassava peel, SEM: indicate standard error of the mean

Conclusion

The result shows that animals fed with 25% and 50% replacement of urea-molasses treated cassava peel ensiled with caged layer droppings showed no significant difference in the heart rate, respiratory rate, earlobe temperature and rectal temperature when compared with that of the control diet. It can be concluded that the different treatments had no effect on the physiology of the animals. The result shows that animals fed with 50% replacement of urea-molasses treated cassava peel ensiled with caged layer droppings gave the highest semen motility, semen live, semen concentration, semen volume, scrotal length, scrotal circumference and libido. It can be concluded that the feed containing 50% replacement of urea-molasses treated cassava peel ensiled with caged layer droppings is best for the animals as it falls within general recommended ranges.

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