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Effects of β-Sitosterol Supplementation on Performance and Antioxidant Activity in Uda Rams

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ABSTRACT

Background and Objective: Uda rams are an important livestock breed in Nigeria; however, their performance and stress resilience under intensive management can be limited. The β-sitosterol, a natural plant sterol, has been reported to possess antioxidant and stress-modulating properties in animals. This study aimed to evaluate the effect of dietary β-sitosterol supplementation on growth performance and antioxidant activity of Uda rams. **Materials and Methods:** The study was conducted at the Usmanu Danfodiyo University Livestock Teaching and Research Farm using a Completely Randomized Design (CRD). Three dietary levels of β-sitosterol, a control and an oxytetracycline check treatment were used. Feed intake and body weight were recorded for 63 days, after which blood samples were collected for the assessment of stress biomarkers and antioxidant activity. The data obtained were analyzed using ANOVA at 5% level of significance. **Results:** No significant differences ($p>0.05$) were observed among treatments for feed intake, average daily gain and feed conversion ratio. Rams supplemented with 1500 g/kg diet exhibited higher ($p<0.05$) total antioxidant capacity (TAC) than those in other treatments, while control and 1000 g/kg β-sitosterol groups showed similar ($p>0.05$) TAC values. Cortisol levels decreased with increasing β-sitosterol levels ($p<0.05$). **Conclusion:** Dietary supplementation of β-sitosterol in Uda rams improved antioxidant status and reduced cortisol levels without negatively affecting growth performance, suggesting its potential as a natural feed additive for stress management in ruminants.

KEYWORDS

β-Sitosterol, growth performance, stress biomarkers, antioxidant activity

INTRODUCTION

Ruminant livestock contribute significantly to Nigeria's economic growth by supplying food for the expanding population and providing vital resources that support national development. They are important in maintaining the livelihood of their keepers by providing food, security, social and cultural identity etc.[1]. However, maintaining them in terms of feeding has been a serious problem of which feed additives might mitigate the problem.

Feed additives are essential in the precision feeding of ruminants, as they help improve feed efficiency and lower the environmental footprint associated with each unit of animal product[2]. In recent years, the use of antibiotics and other growth-promoting substances to enhance production efficiency has become a

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major concern within the livestock industry. This has renewed interest in probiotics as an alternative solution. Additionally, increasing consumer demand for safe, high-quality and nutritious foods has encouraged the adoption of natural feed additives[3]. Consumers generally regard plant-derived phytochemicals as more natural and deem them less toxic feed additives than synthetic chemicals[2].

A multiplicity of phytochemicals has been and are considered for use as natural growth promoters (NGPs) for enhanced livestock productive performance. Phytosterols make up one of the dominant groups of phytochemicals; they are unsaponifiable components of edible vegetable oil products and have a hypocholesterolemia effect. The β-sitosterol is one of the most common phytosterols, which occur either as free-isoforms or as esters of free fatty acids, sugar moieties or phenolic acids[4]. Known to be found in high concentration in *Moringa oleifera* LAM, β-sitosterol can potentially be used as an NGP in livestock production. It has been reported to possess antioxidant, antimicrobial, antibacterial, antifungal and immunomodulatory activities. This study was conducted to evaluate the effects of different levels of β-sitosterol supplementation on the growth performance, stress biomarkers and antioxidant activity of Uda rams.

MATERIALS AND METHODS

Ethical statement: The study was approved by the Institutional Animal Care and Use Committee (IACUC) of Usman Danfodiyo University Sokoto, Nigeria.

Experimental site: The study will be carried out at the Department of Animal Science, Livestock Teaching and Research Farm, Main Campus, Usmanu Danfodiyo University, Sokoto. Sokoto is located between Latitudes 12° and 13° N, Longitudes 4° and 6° E in the Northern part of Nigeria and at an altitude of 350 m above the sea level[4]. The study lasts for 12 weeks (8 March-31 May, 2025).

Experimental animals and management: Twenty-five Uda (yearling weighing average 18-20 kg) rams were used for this study. The experimental animals were sourced from the livestock market within Sokoto State. The animals were dewormed with Ivermectin 5% (1 mL/50 kg body weight) and Albendazole against external and internal parasites, respectively, before the commencement of the experiment.

Also, multivitamins injection was given at a rate of 1 mL/10 kg body weight for three days for any subclinical infections and to reduce stress, respectively. Feed and water were given to the animals *ad libitum*.

Treatments and experimental design: The experiment was conducted in a Completely Randomized Design (CRD) with four treatments and four replications. Each animal serves as a replicate. The animals were acclimatized to their micro-climate for 14 days, provided with feeding and water troughs. The rams were fed *ad libitum*. Test ingredient β-sitosterol, as a natural growth promoter, was offered via wheat offal at 50 g/head/day, which serves as a carrier.

- **Treatment 1:** Serves as a control and will be without supplementation
- **Treatment 2:** Was given 500 mg/kg of β-sitosterol
- **Treatment 3:** Were given 1000 mg/kg of β-sitosterol
- **Treatment 4:** Were given 1500 mg/kg of of β-sitosterol
- **Treatment 5:** Were offered oxytetracycline based on the manufacturer's recommendation

The feeding trial lasted for conducted for 9 weeks (63 days).

Composition of basal experimental diets: Table 1 presents the composition of basal diets fed to.

Table 1: Composition of basal diet fed to Uda rams

Treatment	
Ingredients	Percentage
Wheat offal	20
Maize stover	15
Cottonseed cake	17.1
Rice offal	25.6
Cowpea husk	18.3
Molasses	04.0
Total	100
Calculated chemical composition	
Energy (kcal/kg metabolizable energy)	2509
Crude protein (%)	12.14
Crude fibre (%)	23.46

Data collection

Feed consumption/intake and body weight: Feed consumption from each treatment was measured on a daily basis by subtracting the leftover from the feed served the previous day per group. Adequate measures were taken to guard against spillage and related wastage. Rams were weighed on a weekly basis using a weighing balance to determine the body weight gain. Feed conversion ratio was calculated.

Blood sample collection: The blood was collected from the jugular vein (10 mL) into the vacuum tubes Venoject®, which were then kept in a water bath at 37ºC. Afterwards, serum was isolated by a 10 min centrifugation (3000 revolutions/min) and frozen at -20ºC until assayed.

Stress biomarkers

Hydro cortisol: Blood samples (10 mL) were collected in heparinized vacuum tubes from the jugular vein immediately before the slaughter to determine plasma cortisol concentrations. Hormone concentration was determined by a competitive enzyme immunoassay kit for cortisol determination (Radim, Pomezia, Italy). Validation for ovine plasma was performed as described[5].

Prolactin: Prolactin concentrations were assayed by RIA. The assay sensitivity was set at 2 ng/mL and the intra- and interassay CVs were 8.8 and 11.5%, respectively.

Malondialdehyde-MDA: The changes in malondialdehyde (MDA) levels in serum samples were measured spectrophotometrically.

Antioxidative activity: Total antioxidant capacity (TAC), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in serum were determined using a UV spectrophotometer (T60; PG Instruments, Lutterworth, Leicestershire, UK) with commercially available kits (Sigma-Aldrich, St. Louis, MO, USA).

Data analysis: Data generated from the experiment were subjected to analysis of variance using. Least significant difference (LSD) was used to separate the means at 5% level of significance were applicable.

RESULTS AND DISCUSSION

Performance of Uda rams supplemented with β-sitosterol: The results of the performance of Uda rams supplemented with β-sitosterol are presented in Table 2. The result indicated no significant difference ($p>0.05$) between treatment means in terms of feed intake, average daily gain and FCR.

The effect of β-sitosterol on the feed utilization of Uda rams in this study found no significant differences in growth parameters among treatment groups. The study contradicts the results of Chauhan *et al.*[6] studied that antioxidant supplementation influences performance and short-term heat stress on lamb growth.

Table 2: Performance of Uda rams supplemented with β-sitosterol

	Treatment						
Parameter	Control	500 g/kg	1000 g/kg	1500 g/kg	OxyT	SEM	p-value
Average daily feed Intake (kg)	1.54	1.46	1.45	1.37	1.38	0.65	0.782
Initial body weight (kg)	24.67	24.50	24.50	24.40	24.75	1.85	0.298
Final body weight (kg)	35.96	38.50	36.75	38.00	35.75	2.20	0.087
Weight gain (kg)	11.29	14.00	12.25	13.60	11.00	1.28	0.071
Average daily weight gain (g/day)	179.21	222.22	194.44	215.87	174.60	20.83	0.062
Feed conversion ratio	8.59	6.57	7.46	6.35	7.90	0.89	0.058

Means bearing different superscripts within the same row differ (p<0.05) and SEM: Mean standard error of means

Table 3: Effects of β-sitosterol supplementation on antioxidative activity of Uda rams

	Treatment						
Parameter	Control	500 g/kg	1000 g/kg	1500 g/kg	OxyT	SEM	p-value
TAC (mmoL TE/L)	1.48^{b}	1.35^{c}	1.53^{b}	1.68^{a}	1.38^{c}	0.026	0.0328
SOD (nkat/mL)	0.62^{a}	0.67^{a}	0.61^{a}	0.68^{a}	0.36^{b}	0.025	0.0221
GPx (*)	3.67	3.75	3.87	4.05	3.29	0.196	0.0910

abc: Means bearing different superscripts within the same row differ (p<0.05), SEM: Mean standard error of means, *: nmol NADPH+H/min/mg protein and OxyT: Oxytetracycline

Effects of β-sitosterol supplementation on antioxidative activity of Uda rams: Table 3 shows the effects of β-sitosterol on antioxidative activity in Uda rams. The parameters measured are total antioxidant capacity (TAC), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity.

Except for GPx, all parameters are significantly (p<0.05) across the treatment group. The SOD activity was observed for rams in control, 500 g, 1000 and 1500 g/kg β-sitosterol supplemented group were similar (p>0.05) but higher (p<0.05) than rams supplemented with oxytetracycline.

Rams supplemented with a 1500 g/kg diet had higher (p<0.05) TAC compared to rams in other treatments. The results further showed that rams in control and those supplemented 1000 g/kg β-sitosterol have similar (p>0.05) TAC and higher (p<0.05) than rams supplemented with 500 g/kg and those supplemented with oxytetracycline. Rams supplemented with 500 g/kg and those supplemented with oxytetracycline have similar (p>0.05) TAC.

The significant increase in TAC as the level of β-sitosterol increases contradicts the findings of Tsiplakou *et al.*[7], which suggest that prolonged antioxidant supplementation may lead to a compensatory downregulation of endogenous antioxidant enzymes. The significant reduction in SOD oxytetracycline group indicates that dietary antioxidants are effectively preventing the excessive generation of free radicals, thus reducing the need for high SOD activity, aligning with findings by Tsiplakou *et al.*[7]. The non-variation in GPx activity in might suggest reduced lipid peroxidation more effectively in all the treatment groups. This supports the notion that antioxidants such as β-sitosterol enhance oxidative stress management.

Effects of β-sitosterol supplementation on stress biomarkers of Uda rams: The effects of β-sitosterol supplementation on stress biomarkers was presented in Table 4. The results showed significant variation (p<0.05) in cortisol and T4 levels, while prolactin T3 and MDA remain similar (p>0.05) between the treatment groups.

Rams in control had higher (p<0.05) cortisol levels compared to the other treatment groups, the results further showed that the cortisol levels decrease with increasing levels of β-sitosterol (p<0.05), rams supplemented (p<0.05) oxytetracycline had similar cortisol levels to rams supplemented 1000 and

Table 4: Effects of β-sitosterol supplementation on Stress biomarkers of Uda rams

	Treatment							
Parameter	Control	500 g/kg	1000 g/kg	1500 g/kg	OxyT	SEM	p-value	Reference value
Cortisol (nmol/L)	102.80^{a}	96.32^{b}	83.40^{c}	76.67^{d}	80.29^{dc}	1.98	0.039	42-82**
Prolactin (ng/mL)	67.84	57.30	58.57	49.22	56.67	6.36	0.283	54.5-61.7*
T3 (ng/mL)	1.28	1.76	1.48	2.00	1.63	0.45	0.964	1.08-4.06*
T4 (pg/µL)	62.76^{a}	50.29^{b}	50.53^{b}	48.50^{b}	55.66^{ab}	2.87		47.2-79.8*
MDA (nmol/mL)	2.63	2.83	2.75	2.66	2.81	0.75	0.6441	

abc: Means bearing different superscripts within the same row differ ($p<0.05$), SEM: Mean standard error of means, T3: Triiodothyronine, T4: Thyroxine, MDA: Malondialdehyde **: Jackson and Cockcroft[8] and *: Jung *et al.*[9]

1500 g/kg β-sitosterol but lower cortisol levels than rams in 500 g/kg group. The T4 was also higher ($p<0.05$) in the control than the β-sitosterol-supplemented groups. Rams supplemented with oxytetracycline had similar ($p<0.05$) to rams in the control and β-sitosterol supplemented group. The study found that the level of β-sitosterol had no effect ($p>0.05$) on T4 of Uda rams.

Cortisol, a primary stress hormone, was significantly elevated in the control group (102.80 nmol/L) and decreases with increasing levels of β-sitosterol. This increase may indicate an enhanced adrenal response to stress, potentially due to β-sitosterol role in modulating the hypothalamic-pituitary-adrenal (HPA) axis. However, the cortisol levels remained within the reference range of 42-82 nmol/L except for control and 500 g/kg supplemented groups, suggesting a balanced stress response in the supplemented treatments. And it reveals the stress modulatory effects of treatment against the control. These findings align with previous research indicating that antioxidant supplementation can influence cortisol levels Jung *et al.*[9].

The study observed non-significant differences in T3 levels across treatments, with values ranging from 1.28 to 2.00 ng/mL, within the reference range of 1.08-4.06 ng/mL. Conversely, T4 levels showed significant variation, with lower values observed in β-sitosterol supplemented groups. This decline suggests that B-sitosterol supplementation may influence thyroid hormone metabolism, potentially by enhancing peripheral conversion of T4 to T3, thereby reducing circulating T4 levels. Similar effects have been reported in other studies[10].

CONCLUSION

The study concludes that supplementation of β-sitosterol in the diet of Uda rams does not significantly influence the growth performance. Cortisol and TAC were improved with supplementation of β-sitosterol. The study recommends the supplementation of β-sitosterol, especially at 1500 g/kg in the diets of Uda Rams.

SIGNIFICANCE STATEMENT

This study discovered the beneficial role of dietary β-sitosterol in enhancing antioxidant capacity and reducing stress hormones in Uda rams, which can be valuable for improving animal welfare under intensive production. This study will help researchers uncover critical areas of stress physiology and natural feed additives that many were unable to explore. Thus, a new theory on phytosterol-based stress modulation may be arrived at.

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