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SPERM CHARACTERISTICS AND ANTIOXIDANT INDICIES OF RABBIT BUCKS FED DIETARY L-SELEMETHIONINE AND D-α-TOCOPHEROL

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Abstract

This study evaluated the synergistic effects of dietary L-Selenomethionine and D-α-Tocopherol supplementation on antioxidant indices, and sperm characteristics in rabbit bucks. Twenty 6–7-week-old male weaner rabbits were randomly selected to four dietary treatments: T1 (Control: basal diet only), T2 (basal diet + L-Selenomethionine), T3 (basal diet + D-α-Tocopherol), and T4 (basal diet + both L-Selenomethionine and D-α-Tocopherol). After 12 weeks, antioxidant indices, and sperm characteristics were assessed. Data were analyzed using One-Way ANOVA following the procedures of SAS (2006), and significant means were separated using Duncan's Multiple Range Test (DMRT). Results showed a significant ($p < 0.05$) reduction in malondialdehyde (MDA) levels and a significant ($p < 0.05$) increase in superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) activities in all supplemented groups. Sperm concentration, motility, viability, and morphology also significantly improved ($p < 0.05$), while sperm abnormalities and mortality decreased. The combination of L-Selenomethionine and D-α-Tocopherol (T4) yielded the best outcomes, indicating their potential synergistic role in mitigating oxidative stress and enhancing reproductive performance in rabbit bucks.

Keywords: Catalase, Malondialdehyde, Rabbits, Sperm

Description of Problem

Reproductive efficiency in rabbit bucks plays a vital role in meat production and genetic improvement. However, oxidative stress, as a result of excessive reactive oxygen species (ROS) poses a major threat to testicular health, sperm integrity, and overall fertility. This is particularly concerning in male rabbits, whose spermatozoa are highly sensitive to oxidative damage due to their lipid-rich membranes and limited antioxidant defense (1). Nutritional antioxidants such as Vitamin E and Selenium, especially in its organic form L-Selenomethionine, have garnered attention for their role in counteracting oxidative stress. Vitamin E prevents lipid peroxidation, while Selenium supports the activity of antioxidant enzymes like glutathione peroxidase, helping to maintain testicular and sperm function (2, 3). Studies by Onoja *et al.*, (4) and Al-Rifaie *et al.*, (5) have shown that dietary supplementation with these antioxidants improves sperm quality and reduces oxidative markers. This study, therefore, investigates the individual and synergistic effects of dietary L-Selenomethionine and D-α-Tocopherol on sperm characteristics and antioxidant indices in rabbit bucks, aiming to enhance fertility through nutritional strategies.

Materials and Methods

Experimental site

The experiment was carried out at the Rabbit Production and Research unit, Teaching and Research Farm of the Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

Diet preparation and Experimental treatments

Twenty (20) weaned male rabbits (6-7 weeks old) were balanced for weight and allotted to four (4) treatment groups of five (5) rabbits each in a Completely Randomized Design (CRD). Diets were formulated to contain 16% crude protein

and approximately 2400 Kcal/kg Metabolizable energy (Table 1). Four treatments diets denoted as **T1, T2, T3** and **T4** were compounded.

T1: Control (Basal diet without any supplementation of L-Selenomethionine (Se) and D-α- tocopherol (Vitamin E); **T2:** Vitamin E (basal diet +250 mg of D-α- tocopherol/kg diet); **T3:** Se (basal diet +0.3 mg of L-Selenomethionine /kg diet); **T4:** Vitamin E+Se (basal diet +250 mg of D-α- tocopherol /kg diet + 0.3 mg of L-Selenomethionine /kg diet). Dosage of the supplements is as described by Ebeid *et al.,* (6)

Table 1: Gross composition of the basal diet

Feed Ingredients (%)	Basal diet
Maize	32.61
Soybean meal	16.39
Brewery dry grain	15.00
Rice husk	30.00
Fish meal (72%)	3.00
Oyster shell	2.00
Bone meal	0.25
Vitamin premix*	0.25
Salt	0.25
Lysine	0.15
Methionine	0.10
TOTAL	**100.00**
Calculated Nutrients	
CP (%)	16.20
ME** (Kcal/kg)	2335.76
CF (%)	13.75

*Vitamin Premix: Supply per kg diet: 2 000 000 iu vit. A; 400 000 iu D_3; 8.0 g vit. E; 4 g vit. b_1; 1.0 g vit. B_2; 0.6 g vit.; 0.4 mg vit. B_{12}; 24.0 g Niacin; 0.2 g Folic acid; 8.0 g Biotin; 48.0 g Choline; 320.0 g BHT; 16.0 g Manganese; 8.0 g iron; 7.2 g] Zinc; 0.32 copper; 0.25 iodine; 36. 0 mg cobalt; 16.0 mg selenium. ** ME: Metabolizable Energy calculated using Pauzenga, (7).

Source of supplements

Vitamin E in the form of D-α- tocopherol, an organic source of vitamin E and Organic Se in form of L-Selenomethionine (L-SeMet) was procured from Thorne Research, USA

Data Collection

Antioxidant indices

At the end of the 12–weeks experiment, antioxidant indices (Catalase, glutathione peroxidase, malondialdehyde and superoxide dismutase) were collected into the plain bottles. The protocol for the assays (testosterone and antioxidant indices) was according to the method described for the kits (Elabscience® ELISA Assay, USA).

Sperm analysis

The semen was collected via the caudal epididymis according to the procedure described by Mesbah *et al.,* (8). The sperm count (round and elongated spermatids) and morphology was determined as described by Seed *et al.,* (9). Live sperm and sperm motility was determined using the procedures of Bjorndahl *et al.,* (10) and Cheng *et al.,* (11) respectively.

Statistical analysis

The collected data were subjected to One-way Analysis of Variance (ANOVA) using the Statistical Analysis System (12), and means were separated using Duncan's multiple range test (DMRT) from the same statistical package.

Results

The results presented in Table 2 show that there was a significant ($p < 0.05$) reduction in malondialdehyde (MDA) levels and a significant ($p < 0.05$) increase in SOD, GSH, and catalase (CAT) in supplemented groups compared to the control group (T1).

Table 2: Antioxidant indices of rabbit buck fed diet supplemented with L-selenomethionine and D-α-tocopherol

Parameters	T1(Control)	T2(Vit. E)	T3(Se)	T4 (Vit.E+Se)	SEM
MDA (nmol/ml)	2.28 [a]	0.98 [b]	0.94 [b]	0.95 [b]	0.13
SOD (µmol/ml)	5.81 [b]	7.93 [a]	7.92 [a]	7.93 [a]	0.21
GSH (µmol/ml)	0.25 [b]	0.59 [a]	0.59 [a]	0.58 [a]	0.03
CAT (µmol/ml)	0.24 [b]	0.78 [a]	0.78 [a]	0.78 [a]	0.05

[abc] means on same row with different superscripts differed significantly (P<0.05)

SEM – Standard Error of Mean, *MDA – Malondialdehyde, SOD – Superoxide dismutase, GSH – Glutathione peroxidase, CAT - Catalase

The results presented in Table 3 show that sperm concentration, motility, percentage of normal sperm, and spermatid counts significantly increased ($p < 0.05$) in T2, T3, and T4 compared to the control (T1), while sperm abnormalities and percentage dead were significantly reduced ($p < 0.05$).

The results of this study demonstrated that dietary supplementation with L-Selenomethionine and D-α-Tocopherol significantly improved antioxidant status and sperm quality in rabbit bucks. Reduced levels of malondialdehyde (MDA) and increased activities of superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) across the treatment groups indicate effective mitigation of oxidative stress. These findings are consistent with the reports of Johnson *et al.,* (1), and Naseer *et al.,* (3) the protective role of vitamin E and selenium against lipid peroxidation and cellular damage was revealed. Similarly, Asebe *et al.,* (13) noted improved physiological indices and antioxidant enzyme activities following vitamin E supplementation.

Correspondingly, sperm characteristics such as concentration, motility, viability, and morphology were significantly enhanced, while the percentage of abnormal sperm decreased. These results align with those of Onoja *et al.,* (4) and Fadl *et al.,* (14), observed marked improvements in semen quality following antioxidant supplementation. Al-Rifaie *et al.,* (5) reported that the combination of vitamin E and selenium may not always provide additional benefits beyond individual use. However, the present findings support their combined efficacy in improving both oxidative balance and reproductive parameters under the conditions of this study.

Table 3: Sperm characteristics of rabbit buck fed diet supplemented with L-selenomethionine and D-α-tocopherol

Parameters	T1 (Control)	T2 (Vit. E)	T3 (Se)	T4 (Vit.E+Se)	SEM
Sperm Concentration	71.80 [b]	91.40 [a]	91.60 [a]	91.40 [a]	1.98
Percentage motile	71.76 [b]	86.23 [a]	86.29 [a]	86.76 [a]	1.48
Percentage non motile	28.24 [a]	13.77 [b]	13.71 [b]	13.24 [b]	1.48
Percentage Normal	74.28 [b]	88.06 [a]	88.21 [a]	88.01 [a]	1.39
Percentage Abnormal	25.72 [a]	11.94 [b]	11.79 [b]	11.99 [b]	1.38
Percentage Live	82.96 [b]	8.82 [a]	86.14	85.89	9.38
Percentage Dead	17.04 [a]	14.18 [b]	13.86	14.11	0.38
Round Spermatid	64.50 [b]	98.00 [a]	96.60 [a]	97.60 [a]	3.24
Elongated Spermatid	59.26 [b]	97.40 [a]	98.80 [a]	98.00 [a]	3.88

[abc] means on same row with different superscripts differed significantly ($P<0.05$) SEM – Standard Error of Mean

Conclusion and Application

It could be concluded from this study that dietary supplementation with L-Selenomethionine and D-α-Tocopherol significantly enhances antioxidant activity and improves sperm quality in rabbit bucks. It is recommended that L-Selenomethionine and D-α-Tocopherol be considered as dietary additives for improving reproductive performance in rabbit production systems. However, further research should explore optimal dosage combinations, long-term effects, and possible sex-specific responses to antioxidant supplementation.

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