Journal of Animal Science and Veterinary Medicine
Volume 11(2), pages 138-144, April 2026
Article Number: 2595BA8311
ISSN: 2536-7099
https://doi.org/10.31248/JASVM2026.654
https://integrityresjournals.org/journal/JASVM
Full Length Research

Comparative effects of Vitamin E and garlic oil supplementation on oxidative stress, hepatic and renal in weaner rabbits

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Received Date: 02 April 2026 | Accepted Date: 28 April 2026 | Published Date: 30 April 2026

ABSTRACT: This study evaluated the comparative effects of vitamin E and garlic oil supplementation on oxidative stress markers, hepatic, and renal functions in weaner rabbits. Twenty-four male rabbits (5 weeks old) were used. Prior to the experiment, the housing was disinfected with hypochlorite solution and left to dry for a week before introducing the rabbits into the house. Following a two-week acclimatization period, the rabbits were randomly assigned to three treatment groups (n = 8) in a Completely Randomized Design: T1 (control), T2 (vitamin E at 300 mg/kg body weight), and T3 (garlic oil at 300 mg/kg body weight). Treatments were administered orally for four weeks, and all rabbits were fed commercial grower's mash ad libitum. Oxidative stress biomarkers assessed included glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). Liver and kidney function indices measured were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and creatinine. Results revealed significant differences ($p<0.05$) among treatments. GSH levels were significantly higher in T2 and T3 compared to T1, with no difference between T2 and T3. GSH-Px, CAT, SOD, and MDA differed significantly across all groups. Vitamin E exhibited superior antioxidant effects, including greater reduction in MDA. However, garlic oil showed better improvement in hepatic and renal parameters, recording lower ALT, AST, ALP, BUN, and creatinine values. In conclusion, both vitamin E and garlic oil enhanced antioxidant status and reduced oxidative stress in weaner rabbits. Vitamin E demonstrated stronger antioxidant capacity, while garlic oil was more effective in supporting liver and kidney functions.

Keywords: Alanine aminotransferase, aspartic aminotransferase, blood urea nitrogen, catalase, glutathione, and superoxide dismutase.

INTRODUCTION

Rabbit is scientifically known as *Oryctolagus cuniculus,* and it is economically useful for its meat and fur. For the rabbit to thrive, grow and breed, the biochemical reactions in it must be functioning optimally. This implies that the levels of free radicals and their negative intracellular activities should be low at all times (Ezejiofor *et al*., 2021) Vitamin E (alpha-tocopherol) is a powerful antioxidant showing evidence of affecting physiological activities like digestion, reproduction, growth and immune response in animals (Johnson *et al*., 2023). It does this in the following ways: first, it promotes the synthesis of immunoglobulins which improves the body's resistance to disease, thereby reducing mortality rate among rabbits. Secondly, it promotes healthy cell metabolism by fighting oxidative damage of creatinine kinase during periods of excessive heat because of its lipid solubility and easy transport across cell membranes, thirdly it reduces the cortisol level in the serum, which in turn reduces adverse reactions like

poor feeding and mating of rabbits. This simply means that reproduction is highly aided when alpha-tocopherol is administered to rabbits.

Similarly, garlic oil, which is the liquid extract from garlic (*Allium sativum*), contains a sulfur compound known as diallyl disulfide, which is responsible for its pungent smell (Huang *et al.*, 2023). Other constituents include allicin, alliin, diallyl sulfide, diallyltrisulfide, ajoene, S-allyl-cysteine, saponins and phenols. Together, these compounds have antioxidant, anti-inflammatory, antibacterial, antifungal, neuroprotective, hepatoprotective (liver protection), renal (kidney) protective and immunomodulatory properties (Huang *et al.*, 2023).

Asebe *et al.* (2020) showed that consumption of animal protein is necessary for meeting the protein requirements of humans. Weaner rabbits offer a viable option for meat production and consumption. However, the presence of free radicals in weaner rabbits has the potential of causing various organ and systemic malfunctions, sterilisation, and even a high mortality rate, where the serum antioxidant enzyme levels are quite low; therefore, adding supplements that furnish antioxidant properties in their feed or water is important. Vitamin E and garlic oil contain antioxidant properties, and as such, we tend to compare the potency of Vitamin E and garlic oil on antioxidant enzymes, oxidative stress biomarkers and their effect on the liver and kidney of weaner rabbits. Comparing vitamin E and garlic oil will help clarify rabbit farmers which mechanism is more effective in the tropics.

The objective of this study is to compare the effect of garlic oil and vitamin E on antioxidants (glutathione, glutathione peroxidase, superoxide dismutase and catalase) and pro-oxidant (malondialdehyde) in the tropics. Also, the potency of garlic oil and vitamin E will be compared on liver biomarkers, such as alanine aminotransferase, aspartic aminotransferase, and alkaline phosphatase, and kidney biomarkers, such as blood urea nitrogen and creatinine, respectively.

MATERIALS AND METHODS

Experimental site

The research was carried out at the Teaching and Research Farm of Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria. The research farm is located on a site 18 m above sea level, at a longitude of 4.8062°E and a latitude of 6.9864°N.

Experimental animals and management

The rabbits used for the study were of the New Zealand White breed and were obtained from Umberik International Farm located in Port Harcourt, Nigeria.

Experimental diet

The growers' mash utilized in this study was sourced from the same facility where the experimental rabbits were obtained. The feed was a commercially branded product (TopFeeds), manufactured by Premier Feed Mills Co. Ltd., a subsidiary of Flour Mills of Nigeria Plc. Synthetic vitamin E (alpha-tocopherol) capsules and garlic (*Allium sativum*) oil capsules were procured from Riverside Pharmacy Limited, Akar Road, Rivers State, Nigeria. The vitamin E supplement (Wellspring) was produced in the United States of America, while the garlic oil capsules were manufactured by Nkoyo Pharmaceuticals, Nigeria.

Prior to the commencement of the experiment, the rabbit housing facility was thoroughly cleaned using detergent and subsequently disinfected with a hypochlorite solution one week before animal introduction. The facility was then allowed to air-dry under hygienic conditions. Upon arrival, the rabbits were individually weighed using a calibrated scale to determine their initial body weights, after which they were subjected to a two-week acclimatization period. During this period, the animals were fed growers' mash supplemented with *Centrosema pubescens*.

Experimental procedure

Twenty four (24) male New Zealand white weaner rabbits at 5 weeks old with an average weight of 1.3 kg were randomly selected and allocated to 3 experimental treatments, with each treatment having 8 rabbits (one rabbit per replicate).

Dosage

Vitamin E and Garlic oil were administered to the rabbit orally in a dosage of 300 mg/kg body weight daily via drinking water. For antioxidant experimental studies, the range of vitamin E oral supplementation ranges from 100 – 300 mg/kg body weight (Trombetti *et al.,* 2022). A 3 ml syringe was used to measure the quantities and later converted to mg. 300 mg of vitamin E was administered orally in 150 ml of drinking water daily to each rabbit receiving vitamin E, while garlic oil group received 300mg of garlic oil in 150 ml of water daily. This was done for 28 days.

Sample collection

The experiment lasted for 28 days, and at the end of the period, blood samples were extracted via the jugular vein using a 2 ml syringe and placed in well-labelled non-ethylene diamine tetra acetic acid vial tubes for analysis.

Parameters analyzed and method of analysis

The evaluated antioxidant parameters included reduced glutathione, glutathione peroxidase, superoxide dismutase, and catalase, while malondialdehyde was assessed as an index of lipid peroxidation. Liver function was determined through the analysis of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, whereas kidney function was evaluated using blood urea nitrogen and creatinine levels. Reduced glutathione was quantified following the method of Ellman (1959), and glutathione peroxidase activity was determined according to Paglia and Valentine (1967). Superoxide dismutase activity was measured using the procedure of Misra and Fridovich (1972), while catalase activity was assessed based on the method described by Aebi (1984). Malondialdehyde concentration was determined using the assay developed by Ohkawa *et al*. (1979). The activities of alanine aminotransferase and aspartate aminotransferase were analyzed using the method of Reitman and Frankel (1957), whereas alkaline phosphatase activity was determined following Bessey *et al*. (1946). Blood urea nitrogen and creatinine concentrations were measured according to the methods of Fawcett and Scott (1960) and Bonsnes and Taussky (1945), respectively.

Data analysis

Data obtained were subjected to Analysis of variance (ANOVA) using the general linear model procedure. Treatment means were separated using Duncan Multiple Range Test using statistical software (SPSS, 2016). The experimental Designed employed was Completely Randomized Design. The linear model used in this study is stated below:

$Xij = u + Ti + Eij$

Where: Xij = Value of any observation, u = unknown constant, the population mean common to all treatments, Ti = Treatment effect and Eij = Error term

RESULTS

The results for antioxidant and pro-oxidant are shown in Table 1. Glutathione concentration was significantly higher *(*$p<0.05$*)* in rabbits supplemented with vitamin E (3.22 µmol/g) and garlic oil (3.06 µmol/g) compared to the control group (2.58 µmol/g). Glutathione peroxidase activity showed significant differences across all treatments, with the highest value in the control (1.40 µmol/g), followed by vitamin E (1.29 µmol/g), and the lowest in garlic oil (1.11 µmol/g). Superoxide dismutase activity was significantly increased in the vitamin E group (1.92 µ/ml) compared to the control (1.68 µ/ml) and garlic oil (1.79 µ/ml). Catalase activity was highest in the control (25.38 µ/ml), followed by vitamin E (21.28 µ/ml), and lowest in garlic oil (18.48 µ/ml). Malondialdehyde, a marker of lipid peroxidation, was significantly reduced in the supplemented groups, with vitamin E showing the lowest level (0.23 nmol/ml), garlic oil intermediate (0.31 nmol/ml), and the control highest (0.47 nmol/ml).

The effects of vitamin E and garlic oil are shown in Table 2. All liver enzyme activities (AST, ALT, ALP) and kidney function markers (urea and creatinine) were significantly reduced ($p<0.05$) in rabbits supplemented with vitamin E and garlic oil compared to the control. Aspartate aminotransferase decreased from 72.40 IU/L in the control to 60.80 IU/L in the vitamin E and 49.20 IU/L in the garlic oil groups. Alanine aminotransferase (ALT) followed a similar trend, with values of 62.20 IU/L (control), 55.80 IU/L (vitamin E), and 46.20 IU/L (garlic oil). Alkaline phosphatase activity was markedly reduced in the supplemented groups (30.80 IU/L for vitamin E and 29.80 IU/L for garlic oil) compared to the control (58.40 IU/L). Urea concentration decreased from 42.00 mg/dl in the control to 33.00 mg/dl in the vitamin E and 28.60 mg/dl in the garlic oil groups. Creatinine levels were lowest in garlic oil (1.00 mg/dl), intermediate in vitamin E (1.54 mg/dl), and highest in the control (2.30 mg/dl).

DISCUSSION

Glutathione

The result in this study showed that there were significant differences ($p<0.05$) when the control group was compared to the groups that received vitamin E and Garlic oil, respectively. However, there were no significant differences (*$p > 0.05$*) between the vitamin E group and the Garlic group. This suggested that vitamin E and Garlic oil were able to up-regulate the activities of the glutathione system in the rabbits placed in treatment 2 and treatment 3, respectively. This result corresponds with the findings of Johnson *et al*. (2023) where it was reported that vitamin E was able to up-regulate the activities of glutathione when growing pigs were subjected to oxidative stress induced by a crude oil-contaminated diet. This study also corresponds with the study of Saleh *et al*. (2024) where it was reported that garlic oil supplementation in the diets of female Wistar rats improved antioxidant status by increasing glutathione level in the serum, as well as reducing the level of free radical production when rats were induced by type 11 *Diabetis mellitus*. In the comparative effect, the findings of this study indicate that vitamin E and garlic oil exerted comparable effects on glutathione levels, as evidenced by the similarity in their mean values across the treatment groups.

Table 1. The effect of garlic oil and Vitamin E on antioxidant enzymes and pro- oxidant in weaner rabbit**.**

Parameters	(T_1) Control	(T_2) Vitamin E	(T_3) Garlic	SEM	P-value
GSH (µmol/g)	2.58[b]	3.22[a]	3.06[a]	0.09	0.004
GSH-Px (µmol/g)	1.40[a]	1.29[b]	1.11[c]	0.04	0.000
SOD(µ/ml)	1.68[b]	1.92[a]	1.79[c]	0.03	0.01
CAT (µ/ml)	25.38[a]	21.28[b]	18.48[c]	0.77	0.00
MDA (nmol/ml)	0.47[a]	0.23[c]	0.31[b]	0.03	0.00

[abc] means with different superscript across a given row differ significantly ($p<0.05$). SEM = Standard error of mean, GSH = Glutathione, GSH –Px =Glutathione Peroxidase, SOD =Superoxide Dismutase, CAT = Catalase and MDA =Malondialdehyde.

Table 2. Liver Enzymes and kidney biomarkers of rabbits supplemented with Vitamin E and garlic oil.

Parameters	T1	T2	T3	SEM	p-value
AST(IU/L)	72.40[a]	60.80[b]	49.20[c]	2.54	0.00
ALT(IU/L)	62.20[a]	55.80[b]	46.20[c]	1.83	0.00
ALP(IU/L)	58.40[a]	30.80[b]	29.80[c]	3.81	0.00
UREA(Mg/dl)	42.00[a]	33.00[b]	28.60[c]	1.51	0.00
Creat(Mg/Dl)	2.30[a]	1.54[b]	1.00[c]	0.15	0.00

[abc] means within rows with different superscripts are significantly different ($p<0.05$). AST= Aspartic amino transferase., ALT= Alanine amino transferase., ALP = Alkaline Phosphate., Creat = Creatinine.

Glutathione peroxidase

Glutathione peroxidase is an intracellular antioxidant enzyme predominantly located in the cytoplasm of mammalian tissues, including the gastrointestinal tract, where it plays a key role in the detoxification of peroxides. In the present study, GSH-Px activity varied significantly ($p<0.05$) among treatments, with the garlic oil group exhibiting the lowest mean value, followed by the vitamin E group, while the control group recorded the highest level.

The observed reduction in GSH-Px activity in the supplemented groups may be attributed to increased utilisation of the enzyme in the conversion of oxidised glutathione (GSSG) to its reduced, biologically active form (GSH), reflecting enhanced antioxidant activity. This observation is consistent with the reports of Saleh *et al*. (2026) and Johnson *et al*. (2019), who independently documented that both garlic oil and vitamin E modulate GSH-Px activity in broiler chickens and weaner rabbits, respectively.

Furthermore, the comparatively lower GSH-Px level observed in the garlic oil group relative to the vitamin E group may suggest a stronger overall antioxidant effect of garlic oil. This could be associated with its rich content of diverse organosulfur compounds, which confer broad-spectrum antioxidant activity (Seki and Hosono, 2025). In contrast, vitamin E exhibits potent but more specific antioxidant action, primarily functioning as a lipid-phase chain-breaking antioxidant (Burton *et al.*, 2022).

Superoxide dismutase

Superoxide dismutase is an important antioxidant enzyme that catalyses the dismutation of superoxide anion radicals and other reactive oxygen species (ROS), converting them into hydrogen peroxide and molecular oxygen, thereby reducing oxidative damage in biological systems.

In the present study, SOD activity was significantly higher in the vitamin E-treated group compared to the garlic oil group, indicating a stronger stimulatory effect of vitamin E on the enzyme. This enhanced response may be attributed to the more potent antioxidant capacity of vitamin E. Nevertheless, both treatment groups exhibited higher SOD activities relative to the control group, suggesting that both vitamin E and garlic oil effectively enhanced endogenous antioxidant defence mechanisms in the serum. Again, the outcome of the findings of this result on SOD might be associated with the fact that vitamin E is liable to easily donate hydrogen atoms to neutralise lipid radicals faster, and it is embedded in cell membranes (Burton *et al.*, 2022), while garlic oil does not embed in cell membranes.

These findings are consistent with the report of Sabitha *et al*. (2024), who demonstrated that garlic oil supplementation improves antioxidant enzyme activities, including SOD, in obese rats. The present results therefore support the role of both supplements in upregulating antioxidant defences, although vitamin E appears to exert a comparatively greater effect on SOD activity under the

conditions of this study.

Catalase

Catalase (CAT) is a cytosolic antioxidant enzyme that protects cells, tissues, and organs by catalysing the decomposition of hydrogen peroxide into water and oxygen, thereby mitigating oxidative damage (Johnson *et al*., 2019).

In the present study, significant differences ($p<0.05$) were observed among all treatment groups. Both the vitamin E and garlic oil-treated groups exhibited lower CAT activity compared to the control, while the vitamin E group showed higher CAT values than the garlic oil group. This suggests that vitamin E exerted a comparatively stronger modulatory effect on catalase activity, likely due to its greater antioxidant stability and efficiency in reducing peroxide formation, thereby preserving CAT activity (Anwar et *al.*, 2024). In contrast, garlic oil, which contains volatile sulfur compounds, may exhibit lower stability and undergo rapid metabolism, and at certain concentrations may exert mild pro-oxidant effects.

The findings of this study are consistent with those of Johnson *et al*. (2019), who reported significant changes in serum CAT activity in rabbits subjected to vitamin E supplementation under conditions of oxidative stress induced by short-term hypothermia. However, the present results differ from those of Sauvé *et al*. (2024), who observed no significant effect of vitamin E on CAT activity in piglets fed a deoxynivalenol-contaminated diet compared to the control group. Similarly, Saleh *et al*. (2024) reported no significant changes in catalase activity following garlic oil administration in Wistar rats under diabetes-induced oxidative stress.

Malondialdehyde

The MDA test is basically used to determine the degree of oxidative stress in animals. The study of this result showed that there were significant differences across board in all treatment groups, with vitamin E having the lowest mean, followed by the group that received oral administration of garlic. This indicates that the control group might have experienced a slight level of oxidative stress and that vitamin E and the garlic treatment were able to repair or reduce the level of oxidative damage that our tropical environment may have caused the rabbits. This is evidenced in the findings of Johnson *et al*. (2019), and Sabitha *et al.* (2024). The lowest MDA level observed in the vitamin E group, thus, may indicate that vitamin E had a higher antioxidant potency compared with garlic oil (Anwar *et al*., 2024). Vitamin E is a lipid-soluble, chain-breaking antioxidant localised within cellular membranes, where it plays a critical role in protecting polyunsaturated fatty acids from oxidative damage. It functions by interrupting the propagation phase of lipid peroxidation through the donation of hydrogen atoms to lipid peroxyl radicals, thereby neutralising these reactive species and preventing the continuation of oxidative chain reactions. By inhibiting the formation and spread of lipid radicals, vitamin E effectively limits the breakdown of membrane lipids (Burton *et al.*,2022). Consequently, since MDA is a major end product of lipid peroxidation (Ayala, *et al*., 2014), the suppression of this chain reaction by vitamin E results in reduced MDA formation and lower measurable levels of lipid peroxidation in biological systems, whereas garlic oil acts more indirectly and therefore provides comparatively less reduction in MDA.

Liver and kidney biomarkers

The present study evaluated the effects of oral supplementation of garlic oil and vitamin E on hepatic and renal function indices in rabbits, with emphasis on their roles in mitigating oxidative stress. The activities of key liver enzymes—alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase- were significantly reduced in the treated groups, particularly in the garlic oil–supplemented group (T3), compared with the control. This reduction suggests that antioxidant supplementation effectively attenuated hepatocellular damage. In contrast, the elevated levels of AST, ALT, and ALP observed in the control group are indicative of hepatic stress associated with increased oxidative burden, whereas the reduced enzyme activities in the supplemented groups reflect a hepatoprotective effect attributable to decreased oxidative injury (Li *et al*., 2019; Almatroodi *et al.*, 2020; Rehman *et al.*, 2020; Ezejiofor *et al*., 2021).

A similar pattern was observed in renal function markers. The control group exhibited higher concentrations of blood urea nitrogen and creatinine, suggesting impaired renal function and increased physiological stress. Conversely, both supplemented groups recorded significantly lower values, with the garlic oil group (T3) showing the most pronounced reduction. Elevated urea and creatinine levels are generally associated with compromised renal clearance and enhanced protein catabolism, whereas their reduction reflects improved renal integrity and function (Johnson and Diri, 2020; Canki *et al*., 2024). The observed improvement in renal indices may be attributed to the antioxidant properties of the supplements, which reduce oxidative stress within renal tissues (Dopierała *et al.*, 2025).

These findings are consistent with previous reports documenting the antioxidant and organ-protective properties of vitamin E and garlic. Vitamin E is known to stabilise biological membranes and inhibit lipid peroxidation, thereby exerting protective effects on hepatic

and renal tissues under oxidative stress conditions (Ezejiofor *et al*., 2021). Similarly, garlic exerts antioxidant effects through its sulfur-containing bioactive compounds, which scavenge free radicals and reduce oxidative tissue damage, thereby supporting organ function (Seki and Hosono, 2025).

From an applied perspective, these results are relevant to rabbit production systems. The inclusion of natural antioxidants such as garlic oil and vitamin E in rabbit diets may enhance physiological resilience, improve immune competence, and support organ health (Verma *et al*., 2023). This may translate into improved growth performance, reduced disease susceptibility, and enhanced productivity. Furthermore, the observed improvements in hepatic and renal function suggest potential benefits for longevity and reproductive performance, which are economically important in commercial rabbit production systems (Chen *et al*., 2025).

Conclusion

In conclusion, Vitamin E and garlic oil at 300mg/kg body weight were able to have a positive effect on the antioxidant enzymes and pro-oxidant in weaner rabbits; however, Vitamin E at 300mg/kg body weight performed better. They do this by up-regulating antioxidant enzymes such as GSH-Px, SOD and CAT, while simultaneously reducing the pro-oxidant level [MDA]. Garlic oil at 300mg/kg body weight performed better compared to vitamin E in liver and kidney functionality. This was evident by the lowest concentrations of ALT, AST, ALP, BUN and creatinine compared to the group that received vitamin E.

Recommendations

It is recommended that Vitamin E inclusion at 300 mg/kg body weight should be used in improving antioxidant enzymes, while garlic oil should be used at 300 mg/kg body weight to protect and improve hepatic and renal functions in weaner rabbits.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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