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Comparative Effect of Tocopherol Nanoparticles and Tocopherol on Production and Biochemical Parameters in Local Sheep

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Abstract | Tocopherol is a fat-soluble and antioxidant compound required for the proper function of many organs in the body. Nano-E is a potent water-soluble liquid with antioxidants and is important for the immune, heart, nerves, and reproductive functions. A total of 15 lambs with a weight of 35 kg at 16 weeks are used in the experiment by dividing into three groups, each one having five lambs. The animals are housed together in an identical environment and were fed similarly. The first group (G1) was administrated with nano-vitamin E orally. The second group (G2) was administrated vitamin E orally, while the third group (G3) was given normal saline orally. After eight weeks of treatement, Hb, PCV, cholesterol, triglyceride, ALT, teroxide reductase, body weight, and feed conversion ratio are determined biweekly at 2, 4, 6, and 8 weeks. In every experimental week, the results demonstrated that G1 Hb levels were significantly higher than those of G2 and G3. The Hb value increased with time in G1 and G2. PCV was higher in G1 compared to G2 and G3 during all the weeks; PCV increased with time in G1 and G2. Cholesterol levels in G1 showed lower levels as compared with G2 and G3. The triglyceride levels don't reveal significant differences between the groups, although slight changes exist. ALT levels showed decreasing in G1 as compared with G2 and G3 during the study periods. GSH levels showed significant positive differences in G1 compared to G2 and G3. The body weight showed increasing in G1, as compared with G2 and G3 during the study weeks. Feed conversion ratio (FCR) showed significant positive differences with time in G1, and G2, while constant in G3. G1 showed significant differences in FCR values compared with G2 during the study periods. FCR showed significant positive differences in G1 as compared with G2 and G3. We concluded that nano vitamin E causes increasing in Hb, PCV, body weight, FCR, and GSH, while it causes decreasing cholesterol and ALT, more than vitamin E only.

Keywords | Nano- vitamine E, tocopherol, Hb, PCV, ALT, Cholesterol, Body weight, FCR

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INTRODUCTION

Raising sheep is an important component of the food supply chain and the economic well-being of rural families (Karthik *et al*., 2021). Because of its role as an antioxidant inside cells, vitamin E helps shield cellular membranes from the destructive effects of oxidation (Rizvi *et al*., 2014). Tocopherols and tocotrienols are the two groups that makeup vitamin E; each has four isomers. Even though vitamin E is well recognized for its powerful antioxidant qualities, recent research has shown that it also exhibits anti-inflammatory effects. These essential qualities of vitamin E are advantageous in various facets of health, particularly in terms of neuroprotection and cardiovascular, skin, and bone health. Vitamin E is antioxidant and lipid-soluble. According to (Bohm, 2018), it is among the numerous naturally occurring antioxidants used in the vitamin supplements, culinary, cosmetic, and pharmaceutical sectors. The use of nanoparticles as medication delivery devices holds great promise (Zehiroglu and Ozturk, 2019).

Nano-E is a water-soluble and antioxidant vitamin. It plays an important role in immune, cardiovascular, circulatory, neuromuscular, and reproductive functions. Nanoparticles are composed of nanocapsules and nanospheres (Mohd *et al*., 2020). Nano-E is a source of vitamin E that is readily accessible and absorbed due to the incorporation of cutting-edge nanotechnology into its formulation. Because it is readily accessible, its administration may be timed to offer the highest possible level of antioxidant protection to the development of animals (Guterres *et al*., 2007). Nano formulations, whose diameters range from 1 to 100 nm, have been the subject of a significant amount of research in the context of medication administration applications. It has a high solubility, is effective, and is safe to use, despite its tiny size and wide surface area. These, in turn, boost the bioavailability and effectiveness of the pharmaceuticals provided (Alkaladi, 2019).

Several various Nano-formulation procedures have been tried out in an attempt to solve the problem of vitamin E's low bioavailability so that it might be employed in prospective medicinal applications. The loading of vitamin E into Nano particles or vesicles is some of these ways (Patra *et al*., 2018). In veterinary medicine, animal health, and disease treatment, nanotechnology is crucial (Dawood and Kareem, 2020). In light of the many pharmacological qualities of vitamin E, future research must investigate the possibility of using vitamin E Nano formulations to treat other life-threatening conditions, including cardiac disorders. In the presence of oxygen, light, and heat, vitamin E, which functions as an antioxidant, is very susceptible to oxidation (Adi *et al*., 2019; Kandeil *et al*., 2021).

Vitamin E has a broad variety of beneficial qualities, including those of a medicinal and antioxidant nature. Due to the limited amount of vitamin E that is absorbed by the body, it is not widely used in any therapeutic applications. According to the research findings, nanoparticle formulations increase the delivery and effectiveness of vitamin E by increasing the vitamin's absorption, metabolism, and solubility (Mohd *et al*., 2020).

Our work was intended to accomplish the preparation of vitamin E-loaded Nano-capsules supplementation, and the study of the influence of vitamin E and Nano vitamin E on some of the hematological parameters, enzymes, weight, and FCR in the lambs.

MATERIAL AND METHODS

Rams management

This study was carried out in the Field-College of Veterinary Medicine/University of Baghdad. Before the study started, each rams received a number using ear tags and being under veterinarian supervision. Clinical safety is continuously and carefully evaluated by the preventative system. Every preventative measure was taken for all rams, including a 14-day adaptation period to the farm environment and a subcutaneous (s/c) injection of ivermectin (0.5ml/animal) to protect against external parasites. All rams were administered oral doses of the anthelmintic worminex (2ml/animal), which were repeated after two weeks, to protect them against the consequences of internal parasite infection.

Rams: Feeding, care, and treatment

All of the study's animal-related procedures received permission from the local ethics committee for animal utilization protocol (511/P.G in 5/3/2022). This study conducted on 15 Iraqi Awassi rams weighing 35 kg at age of 52 weeks and divided into three groups, housed together under identical conditions (atmosphere and feeding); experiment diet was continued for a period of 60 days in addition to 14 days as adaptation period. Animals were fed a 2.5% concentrated diet of their body weight (Table 1). Rams were kept in semi-open pens and divided into three groups, five for each group, body weight were considered. The first group (G1) administrated Nano-vitamin E orally (9 mg/Kg). The second group (G2) is administrated vitamin E orally (90 mg/Kg) (Musa *et al*., 2018; Hamzah and Dawood, 2024). The third group (G3) is administrated normal saline orally. The experiment spent eight weeks. Hb, PCV, Cholesterol, Triglyceride, ALT, Peroxide reductase, body weight, and feed conversion ratio are determined biweekly at (2, 4, 6, and 8) weeks as shown in Table 1.

Table 1: Composition of concentration diet's ingredients.

Nutritional ingredients	%
Wheat bran	20
Soya bean	10
Barley	48
Corn	20
Salt	1
premix	1
% Total	100

Blood sample

The blood samples are taken from the jugular vein at animal rest to avoid extreme movement. The samples are put in tubes with an anticoagulant. The tubes are transferred carefully to preventing hemolysis.

Vitamin E and preparation of nano-E

The provided substance as follows, Vitamin E (Sigma, France), Polycaprolactone (10.000 MW), poly dl-lactide-co-glycolide (50,000–75,000 MW), lactide:glycolide (Sigma, France).

The vitamin E-loaded Nano depends on the nanoprecipitation method (Fessi *et al*., 1989). We are adding the vitamin E and PCL together in acetone at 30 °C. and adding the organic solution (dropwise), 5 ml for 60 seconds, in the aqueous consisting of the hydrophilic surfactant with stirring at 25 °C.

The Nano capsule suspension transformed the aqueous phase to milky and purplish. Using a rotavapor (Rotavapor, Switzerland), acetone evaporated at 40 °C under lower pressure for 30 min. A total of 35 cc of Nano capsule suspension water was extracted. Triple preparations were made. The formulation was optimized at each phase using mean size, polydispersity index, zeta potential, aggregate presence, and entrapment efficiency. Nanoprecipitation creates vitamin E-loaded nanocapsules at 165 nm with 98% efficiency.

Hemoglobin examination

Sahli's method is a way to determine the hemoglobin in the blood. Sahli's haemoglobinometer test is used to determine hemoglobin content in blood samples by converting hemoglobin into hematin acid and then diluted produces hematin acid color, which matches with the comparator (Olupot *et al*., 2019).

Packed cell volume test

PCV is a test used to measure the ratio of the volume occupied by RBC to the total volume. The capillary blood tubes are centrifuged, then determined ratio by using percentage fraction.

Serum cholesterol and triglyceride examination

Analysis of total Cholesterol, triglycerides, and HDL are using the Kit and Humalyser Spectrophotometry method (Susandari *et al*., 2004).

ALT and GSH examination

Sera were separated from blood after centrifugation for five minutes at 3000 rpm. Then used directly for serum ALT and Peroxide reductase measurements (Bergmeyer *et al*., 1978).

Feed conversion ratio (FCR)

FCR is an indicator for the conventional measure of animal performance, dividing the feed intake weight by animal weight (Knott *et al*., 2003).

Statistical analysis

The data are represented as mean ± SD. Two-way ANOVA and LSD did the comparisons. (0.05) level of probability was used as the significance. SPSS software (27) are used for performing the comparisons.

RESULTS

Heamatological and biochemical parameters

Our results showed that Hb was significant higher in G1 (10.22, 10.34, 10.6, and 10.9) g/dl as compared to G2 (10.02, 10.16, 10.24, and 10.46) g/dl, and G3 (9.84, 9.84, 9.84, and 9.78) g/dl in (2, 4, 6, and 8) as shown in Table 2).

Table 2: Hemoglobin levels in study groups during the periods (gm/dl).

Groups	Periods / weeks			
	2nd	**4th**	**6th**	**8th**
G1	10.22±0.19Aa	10.34±0.16Aa	10.60±0.17Ab	10.90±0.01Ac
G2	10.02±0.08Ba	10.16±0.08Bab	10.24±0.08Bb	10.46±0.11Bc
G3	9.84±0.11Ca	9.84±0.05Ca	9.84±0.15Ca	9.78±0.08Ca
LSD(P<0.05)	0.158			

Similar letters indicate no significant differences, while different letters indicate significant variations capital letters for vertical, lowercase letters for horizontal.

Table 3: PCV percentage in study groups during the periods.

Groups	Periods/ weeks			
	2nd	**4th**	**6th**	**8th**
G1	37.38±0.46Ac	37.66±0.5Abc	38.16±0.57Aab	38.72±0.64Aa
G2	37.14±0.43Ac	37.46±0.47ABbc	37.80±0.57Aab	38.40±0.51Aa
G3	36.40±0.48Ba	36.86±0.48Ba	36.86±0.45Ba	36.84±0.43Ba
LSD(P<0.05)	0.635			

Similar letters indicate no significant differences, while different letters indicate significant variations capital letters for vertical, lowercase letters for horizontal.

The findings showed that PCV value was high in G1 (37.38, 37.66, 38.16, 38.72%) as compared with G2 (37.14, 37.46, 37.8, 38.4%) and G3 (36.4, 36.86, 36.86, 36.84%) during all the periods (2, 4, 6, and 8) weeks. PCV values increase with time (weeks) in G1 and G2. The eighth week in G1 was a higher PCV value (38.72%) as compared with other weeks at a significant level (P<0.05), as shown in Table 3.

Based on our results, cholesterol levels in G1 (112.4, 108.2, 104.6, 101.6) mg/dl showed lower levels as compared with G2 (114, 110.2, 107, 105.8) mg/dl and G3 (115.8, 116.4, 117.6, and 118.4) mg/dl, which showed higher value of cholesterol. The eighth week in G1 showed a lower value as compared with other weeks at a significant level (P<0.05), as shown in Table 4.

In the second and fourth weeks, there were no significant differences in triglyceride levels between (G1 and G2) and the control group. However, in G3 in the sixth week and eighth week, triglyceride levels showed significant positive differences with G1 and G2 at a considerable level (P<0.05). The triglyceride levels don't reveal significant differences among the groups, although there are slight changes among them at a significant level (P<0.05), as shown in Table 5.

The findings included a significant decrease in ALT levels in G1 (10.88, 10.58, 10.26, and 10.08) U/L as compared with G2 (11.06, 10.84, 10.6, and 10.48) U/L, and G3 (11.34, 11.26, 11.3, and 11.2) U/L in (2, 4, 6, and 8) weeks respectively. However, there are no significant differences between G1 and G2 in the second week and fourth week at a significant level (P<0.05), as shown in Table 6.

GSH levels showed positive significant differences in G1 (110.6, 117.2, 121.4, 127.4) U/g as compared with G2 (101.8, 106.4, 111.4, and 117.2) U/g, and G3 (95.6, 95.8, 95.4, 96.2) U/g. GSH levels showed significant positive differences in G2 as compared with G3 at a significant level (P<0.05), as shown in Table 7.

Production parameters

The results reveal that body weight (Kg) is increased in G1 (36.28, 36.38, 37.16, and 37.6) kg, as compared with G2 (35.74, 36.18, 36.76, and 37.2) kg and G3 (control group) (34.9, 35.16, 35.38, and 33.9) kg in (2, 4, 6, 8) weeks respectively. However, there were no significant differences among some of the groups, at a considerable level (P<0.05), as shown in Table 8.

Table 4: Cholesterol levels in study groups during the periods (gm/dl).

Groups	Periods/ weeks			
	2nd	4th	6th	8th
G1	112.4±3.43Aa	108.2±3.42Bab	104.6±4.15Bbc	101.6±3.78Bc
G2	114±4Aa	110.2±1.64Bab	107±2.54Bb	105.8±6.22Bb
G3	115.8±3.76Aa	116.4±4.03Aa	117.6±3.78Aa	118.4±3.04Aa
LSD(P<0.05)	4.83			

Similar letters indicate no significant differences, while different letters indicate significant variations capital letters for vertical, lowercase letters for horizontal.

Table 5: Triglyceride levels (mg/dL) in study groups during the periods.

Groups	Periods/ weeks			
	2nd	4th	6th	8th
G1	40.46±0.05Aab	40.62±0.39Aa	40.26±0.15Bb	40.14±0.11Ab
G2	40.32±0.14Aa	40.32±0.16Aa	40.30±0.15ABa	40.34±0.15Aa
G3	40.58±0.52Aa	40.60±0.46Aa	40.62±0.39Aa	40.46±0.05Aa
LSD(P<0.05)	0.356			

Similar letters indicate no significant differences, while different letters indicate significant variations capital letters for vertical, lowercase letters for horizontal.

Table 6: ALT levels (U/L) in study groups during the periods.

Groups	Periods/ weeks			
	2nd	4th	6th	8th
G1	10.88±0.13Ba	10.58±0.25Bb	10.26±0.32Cc	10.08±0.3Cc
G2	11.06±0.08Ba	10.84±0.11Bab	10.60±0.3Bbc	10.48±0.17Bc
G3	11.34±0.26Aa	11.26±0.11Aa	11.30±0.1Aa	11.20±0.13Aa
LSD (P<0.05)	0.267			

Similar letters indicate no significant differences, while different letters indicate significant variations capital letters for vertical, lowercase letters for horizontal.

Table 7: GSH levels (U/g) in study groups during the periods.

Groups	Periods/ weeks			
	2nd	4th	6th	8th
G1	110.6±2.07Ad	117.2±1.18Ac	121.4±1.18Ab	127.4±1.14Aa
G2	101.8±1.11Bd	106.4±0.18Bc	111.4±1.2Bb	117.2±1.22Ba
G3	95.6±1.23Ca	95.8±1.2Ca	95.4±1.25Ca	96.2±5.05Ca
LSD(P<0.05)	2.88			

Similar letters indicate no significant differences, while different letters indicate significant variations capital letters for vertical, lowercase letters for horizontal.

Table 8: Body weight (Kg) in study groups during the weeks.

Groups	Periods/ weeks			
	2nd	4th	6th	8th
G1	36.28±1.22Aa	36.38±1.18Aa	37.16±1.18Aa	37.6±1.14Aa
G2	35.74±1.11Aa	36.18±0.18Aa	36.76±1.2Aa	37.2±1.22Aa
G3	34.9±1.23Aa	35.16±1.2Aa	35.38±1.25Aa	33.9±5.05Ba
LSD(P<0.05)	2.34			

Similar letters indicate no significant differences, while different letters indicate significant variations capital letters for vertical, lowercase letters for horizontal.

Table 9: Feed conversion ratio (FCR) values in study groups during the periods.

Groups	Periods/ weeks			
	2nd	4th	6th	8th
G1	5.02±0.04Ac	5.18±0.08Ab	5.26±0.05Aab	5.36±0.05Aa
G2	4.84±0.05Bc	4.96±0.08Bbc	5.08±0.16Bb	5.24±0.16Aa
G3	4.62±0.08Ca	4.68±0.08Ca	4.68±0.08Ca	4.68±0.08Ba
LSD(P<0.05)	0.121			

Similar letters indicate no significant differences, while different letters indicate significant variations capital letters for vertical, lowercase letters for horizontal.

According to the study, the Feed conversion ratio showed significant positive differences with time in G1, and G2, while it was constant in G3. G1 showed significant positive differences in FCR values (5.02, 5.18, 5.26, 5.36) in (2, 4, 6, 8) weeks, respectively, as compared with G2 (4.84, 4.96, 5.08, 5.24) in (2, 4, 6, 8) weeks respectively, while the G3 were (4.62, 4.68, 4.68, 4.68) at a significant level ($P<0.05$).

The general average (FCR) values in G1 were higher than G2 and G3 and showed significant differences among the group at a considerable level ($P<0.05$)(Table 9).

DISCUSSION

Our results showed that Hb and PCV had higher significant differences in the group which administrated nano vitamin E than in the control group. The study revealed the effect of vitamin E on FCR, Cholesterol, ALT, AST, PCV, and Hb. The rams fed on the diet with Vit. E showed an increase in FCR, Hb, and PCV. Vitamin E is an important additive to rams' diets to improve productivity (Imran *et al*., 2019). Our results agreed with Dawood and Al-Saigh (2015) who have discovered that vitamin C and vitamin B complex significantly increased PCV, Hb, WBCs, lymphocyte percentage, and decreased neutrophil, monocyte, and eosinophil percentages compared to the control group.

Administration of Vit. E for eight weeks increased ferritin in the serum at a normal level and vitamin E levels increased (Tang *et al*., 2016). There were significant differences in total RBC, PCV, and hemoglobin compared to the control in the treated group that administered vitamin E. Administration of vitamin E were improved the majority of blood's biochemical and immune qualities, as well as its physical state (Abdullah and Hassan, 2021).

Feeding fish with vitamin E with selenium will increase the growth parameters and reduce plasma ammonia in fish, as well as the highest RBC, hemoglobin, and PCV (Nazer *et al*., 2020). Cholesterol levels in the nano vitamin E group decreased in the group that administrated Nano vitamin E compared to the control group. The triglyceride levels don't reveal significant differences among the groups, although there are slight changes among them at a significant level ($P<0.05$).

After administration of vitamin E for eight weeks, there are no significant differences in cholesterol. However, it showed a decrease in high-density-lipoprotein Cholesterol (Leonard *et al*., 2007). Administration of Vitamin E for 28 days will decrease hypercholesterolemia in the rabbits. Vitamin E has a hypocholesterolemic effect (Torres *et al*., 2003).

High levels of serum lipids were prevented by administering vitamin E (Oregon State University, 2015). That explains the results of some studies that showed no significant differences between vitamin E and levels of Cholesterol and Nano vitamin E; furthermore, between triglyceride and vitamin E.

It was found that male rabbits were divided into three groups, and the results showed a decrease in Cholesterol and triglycerides compared with the control group. Vitamin E causes a marked change in the lipid profile (Al-Sahlawi *et al*., 2020).

Recent investigations, however, reveal that vitamin E may possibly exhibit other biological actions. Vitamin E reduces the encoding of Cholesterol biosynthesis pathway enzymes. vitamin E may compart in resulting of the inhibition of cholesterol production (Valastyan *et al*., 2008). vitamin E inhibits fatty acid peroxidation and prevents malondialdehyde production, leading to a decrease in triglyceride synthesis. The tocotrienols in vitamin E composition lowers cholesterol concentration and prevents plaque buildup in the arteries (Haglund *et al*., 1991).

The findings included a significant decrease in ALT levels in the group that administrated nano vitamin E as compared to the control group. The mice fed a vitamin E diet decreased liver enzymes (Pacana and Sanyal, 2012). After the administration of vitamin E, serum ALT concentration will decrease weekly (Hoofnagle *et al*., 2013). vitamin E administration will decrease ALT concentration in patients with liver diseases (Kim *et al*., 2015). Current evidence affirms that vitamin E-whether alone or combined improves biochemical such as (liver enzymes) and histological outcomes in adults and pediatric patients.

Production parameters

According to the study, FCR is increased in the group that administrated Nano vitamin E as compared with the control group. The body weight is increased with the administration of Nano vitamin E compared to the control group. The feeding of vitamin E improvement in Hepatic lipid peroxidation, absolute maximum weight, best feed conversion ratio, and protein retention efficiency in fish fed on a diet supplemented with 140 mg of vitamin E. Also, the study showed improvement in PCV and Hb (Siddiqui and Khan, 2022).

After administration of vitamin E, The immune response was improved, and the lymphocyte and total WBC and immunoglobulins levels were increased in broiler chicken. Furthermore, there are improvements in the feed conversion rate (Pompeu *et al*., 2018). The vitamin E supplementation in the diet of Awassi male lambs will increase the average weight and feed conversion rate (Macit *et al*., 2003; Leal *et al*., 2018).

CONCLUSIONS

Vitamin E has a positive role in hematological parameters such as hemoglobin, hematocrit, body weight, feed conversion rate, and GSH. At the same time, it causes decreased cholesterol and ALT levels in serum. Nano vitamin E has a more significant role than E vitamin.

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NOVELTY STATEMENT

This study provides new insights into the superior effects of Nano-E compared to conventional vitamin E on key health and biochemical parameters in lambs. By assessing the impacts on Hb, PCV, lipid, liver function, antioxidant status, body weight, and feed conversion ratio, this research highlights the enhanced bioavailability and efficacy of Nano-E. The nanoformulation demonstrated significant improvements in hematological indices, antioxidant capacity, growth performance, and reductions in cholesterol and ALT levels, outperforming both traditional vitamin E and control treatments. These findings contribute novel evidence supporting the use of nano-vitamin E as a potent dietary supplement to enhance immune function, metabolic health, and growth in livestock, paving the way for innovative applications of nano-antioxidants in animal nutrition and health management.

AUTHOR CONTRIBUTION

Tamara did all the work related to conducting and completing this research, from conducting the experiment to the final publication of the research.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The author has declare no conflict of interest regarding this manuscript.

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