

## **Influence of Dietary High Level of Antioxidant Vitamins and Zinc Methionine on Performance, Digestibility and Immune Response of Growing Rabbits under Sub-tropical Egyptian Conditions**

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### **Abstract**

The effect of vitamin E ( $\alpha$ -tocopherol), vitamin C (ascorbic acid) and zinc methionine and/or their combination on performance, carcass traits, digestibility and immune response were studied in New Zealand growing rabbits under sub-tropical Egyptian conditions. Fifty weaner New Zealand White rabbits of both sex (age of 30 days and initial weight of  $555 \pm 12.0$ g) were used in this experiment. The growing rabbits were randomly divided into five equal experimental groups of ten each, and submitted to the five dietary treatments. Experimental period extended for about 42 days from weaning (30-day) to slaughter age (72 days), and the last 5 days were assigned for fecal collection. Rabbits in the first group (control) were fed on pelleted basal control ration with no extra vitamins or zinc supplement and the diet was formulated to satisfy the nutritional requirements of growing rabbits. Animals in the second, third and fourth groups fed on the same diet but fortified with 300 mg  $\alpha$ -tocopherol acetate, 4g ascorbic acid and 100mg/kg basal diet, respectively. Growing rabbits in the group 5 fed on the same diet supplemented with mixture of 150 mg  $\alpha$ -tocopherol acetate, 2g ascorbic acid and 50 mg zinc methionine/kg diet. The obtained results indicated that massive addition of vitamin E, C, zinc methionine and their combination improved the total body weight gain by about 9.5, 9.0, 9.4, 11.9 % respectively, matched with substantial reduction in feed conversion by about 7.5, 6.9, 8.4, and 9.7 % than control. Dressing percentage was increased significantly ( $P < 0.05$ ) in the second and fifth groups (58.11 and 58.82 %) compared with control one (56.19%), while no significant differences were observed for weights of fore limb, hind limb, liver and internal organs between the different experimental groups. Dietary supplementation of vitamin E, C and zinc methionine alone or in combination caused insignificant increase in the digestion coefficients of all nutrients and nutritive values, and the fifth group recorded the highest values. Total serum protein and urea significantly ( $P < 0.05$ ) increased in the groups supplemented with zinc, while no significant differences among treatments were recorded for serum cholesterol.  $\alpha$ -tocopherol, ascorbic acid and zinc concentrations were significantly higher in the

serum of rabbits which simultaneously ingested the highest amounts of these nutrients. Significant ( $P < 0.05$ ) increase in the total erythrocytic and leucocytic counts was observed in the fifth group fed on the combination of vitamin E, C and zinc methionine. B and T-cell reactions were increased in all supplemented groups and the best result was noticed in the fifth group which show marked increase of the B-cells reaction especially in the lymph nodes and spleen either in red or white pulp. The results of this study concluded that dietary supplementation levels of vitamin E, C and/or zinc methionine beyond the recommended level improve growth rate and feed efficiency of growing rabbits and co-supplementation of these nutrients confirmed synergistic positive effect on immune response of this animal.

*Key words:* Vitamin E, C, zinc, performance, immune response, growing rabbits.

## **Introduction**

Nutrition has a profound effect on immunity and health in animals and poultry. Vitamin E ( $\alpha$ -tocopherol), vitamin C (ascorbic acid) are naturally-occurring antioxidant nutrients that play important role in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors (15). Dietary supplementation has been proved to be a simple and convenient strategy to introduce a natural antioxidant that may effectively inhibit the oxidation reactions (9). Vitamin E is a highly effective natural antioxidant that protects cellular membranes against oxidative change. Vitamin C is an essential micro-nutrient required for normal metabolic functioning of the body. Ascorbic acid can also reduce the generation of oxidants and regenerates  $\alpha$ -tocopherol from its oxidative form (35; 25 and 38). Dietary supplementation of vitamin E and C at levels higher than the minimum requirements may influence the immune responses and resistance to diseases, thereby increasing overall performance of several animal species. Based on that antioxidant action, both vitamins have been used under stress or un-coming conditions to improve the performance in vivo (15; 38 and 40 in rabbits, and 44 and 25 in calves). In rabbits, (31), (16) and (40) working on vitamin E and (1), (39), (38) and (40) working on vitamin C, they all reported a growth promoting action for two vitamins. Other studies failed to prove such response (13, 12, 11; 34; 17; 9 and 41). (37) and (2) concluded that a combination of higher dietary levels of ascorbic acid and vitamin E had a positive synergistic effect

on the immune response and provided greatest performance in Japanese Quills and broilers. Zinc has both specific and unspecific role on immune defense mechanism. Zinc involved in many biological functions and enhances cell mediated immunity and therefore improves production efficiency in rabbits (4; 19; 47; 22; 5 and 10). The present study was undertaken to evaluate, under field conditions the effect of dietary vitamin E, C, zinc methionine and their combination on performance, carcass traits, digestibility and immune response of growing rabbits.

### **Materials and Methods**

#### **Animals, Management and feeding:**

Fifty weaner New Zealand White rabbits of both sex (age of 30 days and initial weight of 555±12.0g) were used in this experiment. The growing rabbits were randomly divided into five equal experimental groups of ten each, and submitted to the five dietary treatments. All growing rabbits were housed in galvanized wire cages that allowed separation of feces and urine. A cycle of 12 hours of light and 12 hours of dark was used throughout the experiment. Heating and forced ventilation system allowed the building temperature to be maintained between 20 and 30 °C. All rabbits were kept under the same managerial and hygienic conditions and were subjected to a prophylactic vaccination and pharmacological program against viral and bacterial diseases. Experimental period extended for about 42 days from weaning (30-day) to slaughter age (72 days), and the last 5 days were assigned for fecal collection and digestibility determination.

Rabbits in the first (control) group were fed on basal control diet with no extra vitamins and zinc supplement (10 mg/kg vitamin E ( $\alpha$ -tocopherol acetate and 50 mg zinc/kg diet provided through the vitamin and mineral premix, N.R.C., 33). Animals in the second and third groups fed on the same basal control diet but fortified with 300 mg  $\alpha$ -tocopherol acetate/kg diet and 4 g ascorbic acid/kg diet, respectively. The amount of vitamin E added confirms good resistance of vitamin E to feed processing. In the fourth group zinc methionine was added at the rate of 100mg/kg diet, while rabbits in the group 5 fed on the same basal control diet supplemented with 150 mg  $\alpha$ -tocopherol

acetate, 2g ascorbic acid and 50 mg zinc methionine/kg diet. Basal control diet was formulated in a pelleted form to satisfy the N.R.C. (33) recommendation. Physical and chemical composition of the basal diet is presented in Table (1). To avoid vitamin C oxidation during pelleting process, the vitamin was dissolved in 30 – 50 ml water and then sprayed over the pellets every day. The pelleted basal diet was fed to rabbit's ad-libitum and fresh water was automatically available at all times by stainless-steel nipple for each cage.

**Parameters evaluated:**

During 42-day growth trial period, initial weight was recorded at the weaning age (30-day), live body weight development and feed intake were weekly recorded. Total body weight gain, feed consumption and feed conversion indices were calculated for the whole experimental period from weaning to slaughter age (at 72 days). The amount of feed consumed was divided by the total body weight gain of the rabbit in order to calculate the rate of feed conversion. At the end of the experiment, three rabbits from each group were taken randomly, fastened for 12 hours (27), and then slaughtered. Skin was immediately removed and after complete bleeding (within 30 minutes), pelt, viscera, distal portion of legs and tail were removed to obtain dressed carcass weight. Thereafter, the eviscerated carcass was cut into fore-limb, hind-limb and loin. These parts were weighed and proportionate to eviscerated carcass weight. Internal organs weights including heart, kidney, liver and lung were calculated as a percentage of eviscerated dressed carcass weight.

**Fecal and blood samples collection:**

During the last 5 days of the experimental period (collection period), feces was quantitatively collected daily from each rabbit. Feces from each animal were weighed, sampled, mixed, dried (60 °C), ground and stored to be analyzed for different nutrients. Two blood samples were collected from each slaughtered rabbit in the five groups at the end of the experiment, one with EDTA and other without EDTA. Blood samples without EDTA were allowed to clot at ambient temperature, centrifuged and sera were separated and kept at -20 °C till further biochemical analysis.

**Analytical methods:**

Basal experimental diet and feces collected were sampled, dried, ground, mixed thoroughly and analyzed for determination of different nutrients according to the method of the AOAC (3) official method.

**Digestion coefficients:**

From the analysis of the basal diet and fecal matter excreted, the digestion coefficients of dry matter and other nutrients were calculated according to the following equation (29).

$$\text{D.C. of any nutrient} = \frac{\text{Amount of nutrient intake} - \text{amount of nutrient in feces}}{\text{Amount of nutrient intake}} \times 100$$

**Serum biochemical parameters:**

The blood serum parameters, including total serum protein, urea, and cholesterol were determined using standard test kits supplied by Biomerieux (Baines / France). Serum  $\alpha$ -tocopherol was determined calorimetrically according to the standard method described by (24), while total serum ascorbate was estimated by the 2, 4-Dinitrophenyl hydrazine method reported by McCormick and Green (30).

**Immunological Assays:**

Blood samples with EDTA were used for the detection of:

- a- Total erythrocytic count/ mm<sup>3</sup> blood
- b- Total leucocytic count/ mm<sup>3</sup> blood.
- c- Differential leucocytic count on blood film stained with Giemsa stain.

**Immunohistochemical studies:**

Specimens were taken from spleen and lymph node, then fixed in cold acetone, processed and paraffin infiltrated. The paraffin blocks were sectioned at 7  $\mu$ . The prepared sections were used for immunologic studies by the following histochemical indices: a- Alkaline phosphatase reaction for detection of activated B-lymphocytes (**Gomeri method, 23**). b- Non specific esterase activity for detection of T-lymphocytes (26).

### **Statistical analysis:**

All data were subjected to one-way analysis of variance (ANOVA) using the general lines model of Statistical Analysis System (36), and differences ( $P < 0.05$ ) among treatments were tested using Duncan's multiple range test (18).

## **Results and Discussion**

### **Growth performance and carcass traits:**

The effect of vitamin E ( $\alpha$ -tocopherol), vitamin C (ascorbic acid) and zinc methionine and/or their combination on performance, carcass traits, digestibility and immune response were studied in New Zealand growing rabbits under sub-tropical Egyptian conditions. Data on growth performance in terms feed intake, body weight development, live body weight gain and feed conversion are presented in tables 2, 3 and 4. Mean values cleared that rabbits had access to extra levels of vitamin E, C and zinc methionine beyond recommendation levels achieved significantly ( $P < 0.05$ ) better growth performance compared with control group. Values indicated that treatment with vitamin E or C and their combination with zinc methionine caused a slight increase in feed intake as compared to control. Massive addition of vitamin E, C, zinc methionine and their combination improved the total body weight gain by about 9.5, 9.0, 9.4, 11.9% respectively, matched with substantial reduction in feed conversion by about 7.5, 6.9, 8.4, and 9.7 % than control. The minimum values for both variables were belonged to the ascorbic acid group (gain 1195g and conversion 2.98); while the superiority was for the fifth group (gain 1226g and conversion 2.89). Favorable effect of the combination of three supplements on rabbit performance in the fifth group may be due to a positive synergistic effect on the immune response which provided greatest performance (37). The effects of  $\alpha$ -tocopherol and/or ascorbic acid on growth performance may be fluctuated due to several factors as initial weight, length of the study, environmental conditions, dose of vitamin studied and way of introduction.

The improvement in live body weight and feed conversion efficiency of growing rabbits fed high levels of vitamin E are in agreement with the findings of (39), (31), (16) and (40) in rabbits, and (46), (45) and (2) in

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broilers. Other reports failed to detect a growth promoting action for vitamin E as (13 and 12), (34), (9) and (17). Also the current results of the group fed diet supplemented with high dose of vitamin C confirms the findings of (1), (39), (38) and (40) who reported that vitamin C supplemented diet resulted in better performance and lower feed conversion in growing rabbits. On the other hand, (41) reported no further response of vitamin C on growth performance in rabbits. The beneficial effects of  $\alpha$ -tocopherol and/or ascorbic acid may be attributed to the enhancement of the total antioxidant status (40). Zinc methionine alone or in a combination with antioxidant vitamins had a significant ( $P<0.05$ ) increase in LBW and feed efficiency. Similar results were obtained by (4), (19), (6), (22), (28) and (5) who found that zinc supplementation increased live weight gain and feed conversion efficiency in rabbits.

Concerning the effect of antioxidant vitamins and zinc supplementation on carcass traits (table, 5), obtained results revealed that only dressing percentage was increased significantly ( $P<0.05$ ) in the second and fifth groups (58.11 and 58.82 %) compared with control one (56.19%). These results are supported by observations of (42), (16) and (40) who concluded that dressing percentage was significantly improved with vitamin E supplementation. Other studies carried out by (13), (39) and (40) reported no effect of vitamin C and/or E on carcass traits. There were no significant ( $P<0.05$ ) differences between different experimental groups for the mean weights of fore limb, hind limb, lion and internal organs including liver, heart, kidneys and lungs expressed as percentages of dressed carcass weight. The same was recorded by (47) and (43) as they found that no effect for zinc supplementation on dressing percentage and internal organ weights in both growing rabbits and male guinea pigs, respectively.

### **Nutrient digestibility:**

The results of nutrient digestibility, table (6) showed that dietary supplementation of vitamin E, C and zinc methionine alone or in a combination had no significant increase in the digestion coefficients of all nutrients and nutritive values including DCP and TDNs. The growing rabbits fed diet supplemented with a combination of vitamins and zinc in the fifth group recorded the highest values for all nutrients. Similar results were

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recorded by (38) who found that ascorbic acid supplementation slightly increased digestibility of all nutrients in rabbits. On the other hand, (19) and (47) reported that rabbits fed diet supplemented with 170 mg zinc/kg diet had an increase in the digestibility of all nutrients. On the contrary, (20) found that, vitamin E had no effect on digestibility coefficients in growing rabbits, and (43) stated that zinc supplementation from different sources had no significant effect on digestibility in male guinea pigs.

#### **Serum biochemical parameters:**

Serum biochemical parameters including total serum protein, cholesterol, urea, vitamin E, vitamin C and zinc are illustrated in table (7). Data indicated that total serum protein and urea significantly ( $P<0.05$ ) increased in fourth and fifth groups supplemented with zinc compared with other treated groups and control, while no significant differences among treatments were recorded for serum cholesterol. These results are in agreement with the studies of (6) and (28) who reported that zinc supplementation significantly increase final body weight, total serum protein and urea-N in rabbits during period of heat stress. (43) found that total serum protein, albumin and alkaline phosphatase activity were higher in all zinc supplemented groups in male guinea pigs. Serum concentrations of  $\alpha$ -tocopherol, ascorbic acid and zinc of all the supplemented rabbits were significantly ( $P<0.05$ ) higher than the control and this agreed with that previously recorded by (25), (12), (22) and (43) who found that ascorbic acid, vitamin E and zinc concentrations in the serum depended on the uptake of these nutrients.

#### **Haematological examination:**

The hematological picture (Table, 8) showed significant ( $P<0.05$ ) increase in the total erythrocytic count and highly significant one in the total leucocytic count in the fifth group fed on diet supplemented with the combination the supplement in comparison with control one. Marked increase in the percentage of lymphocytes in groups 3 and 5 and marked decrease in the percentage of neutrophils in groups 4 and 5. Results on hematology were similar to those reported previously by (39) working on vitamin E, (31) working on vitamin C and (40) working on both two vitamins and their combination in rabbits. They all reported that these vitamins had appreciable



significant effect on lymphocytes percentage which considers a good indicator for increasing immunity efficiency. (7) found that vitamin E plays an important role in protecting leukocytes and macrophages during phagocytosis; it protects leukocytes from the toxic products that produced from ingested bacteria.

#### **Immunohistochemical studies:**

The alkaline phosphatase reaction showed few B-cell reactions (which took blackish color) in both lymph node and spleen (Figs., 1, 3). In the different supplemented groups, the B-cell reaction was increased, wherever the best result was noticed in the fifth group. In this group, there was marked increase of the B-cells reaction especially in the lymph nodes and spleen either in red or white pulp (Figs., 2, 4). This may be attributed to a positive synergistic effect of antioxidant vitamins (8). Non specific esterase reaction is a marker of T-cells revealed few T-cell reactions which took brownish color in the lymph node of control rabbit (Fig., 5). T-cell reaction increased in different supplemented groups especially in group 5 (Fig., 6). The positive effects of vitamin E, C and zinc methionine supplementation on B and T lymphocytes response was reported by (15), (43) and (10). Zinc regulates the maturation and functions of immune cells by protecting developing lymphocytes from apoptosis, and as a part of zinc finger protein and may influence DNA transcription (21). Vitamin C also may function to reduce the tocopheroxyl radical, thereby restoring the radical scavenging activity of vitamin E (32). Vitamin C interacts synergistically with  $\alpha$ -tocopherol at the membrane cytosol interface to generate membrane-bound oxidized vitamin E (32 and 14). Interaction among these antioxidant nutrients are likely very important in protecting cells because the concentration of each antioxidant alone may not be adequate to effectively protect these cells against lipid peroxidation. The results of this study concluded that dietary supplementation levels of vitamin E, C and/or zinc methionine beyond the recommended level improve growth rate and feed efficiency of growing rabbits and co-supplementation of these nutrients confirmed synergistic positive effect on immune response of this animal.

## References

1. **Abdel-Hamid, E. (1994):** Effect of adrenal hormone and ascorbic acid on resistance of growing rabbits. Ph. D. Thesis, Fac. Agric., Alex.Univ., Egypt.
2. **Abdel-Raheem, H.A. and Abdel-Ghaffar, S.Kh. (2004):** Effect of Ascorbic acid, vitamin E, and melatonin on performance, and immune response of broilers. Assiut Vet. Med. J., 50(101): 215-233.
3. **AOAC (1990):** Association of Official Analytical Chemists. Official Methods of Analysis (14<sup>th</sup> Ed.) AOAC, Washington, DC.
4. **Ayyat, M.S. (1993):** Response of weanling rabbits to feeding of zinc bacitracin and flavomycin as growth promoters under Egyptian conditions. Egypt. J. Rabbit Sci., 3(2): 171-177.
5. **Ayyat, M.S. and El-Aasar, T.A. (2008):** Effect of season of the year and dietary zinc supplementation on doe and buck performance of New Zealand White rabbits, under Egyptian conditions. Egypt. J. Rabbit Sci., 18(1): 1-14.
6. **Ayyat, M.S.; Gabr, H.A.; Marai, I.F.M. and Abdel-Monem, U. M. (1997):** Alleviation of heat-stressed growing rabbits by using some chemical growth enhancers, under subtropical Egyptian conditions. International conf. on animal, poultry and rabbit production and health. Egypt. Internat. Centre Agric., Dokki, Cairo, Egypt, 2-4 September, 1997, pp. 637-651.
7. **Badwev, J. and Kamovsky, M. (1980):** Active oxygen species and the function phagocytical leukocytes. Animal Review Biochem., 49:695-726.
8. **Bendich, A.; Apolito, P.D.; Gabriel, E. and Machlin, L.J. (1981):** Interaction of dietary vitamin C and vitamin E on guinea pig immune responses to mitogens. J. Nutr., 114: 588-593.
9. **Botsoglou, N.; Florou-Paneri, P.; Christaki, E.; Giannenas, I. and Spais, A. (2004):** Performance of rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with oregano essential oil. Arch. Animal Nutr. 58 (3): 209-218.
10. **Cardinali, R.; Rebollar, P.G.; Dal Bosco, A.; Cagiola, M.; Moscati, L.; Forti, K.; Mazzone, P.; Scicutella, N.; Rutili, D.; Mugnai, C. and Castellini, C. (2008):** Effect of dietary organic acids and essential oils on immune function and intestinal characteristics of experimentally infected rabbits. In: Proc. 9<sup>th</sup> World Rabbit Congress- June 10-13, 2008, Verona – Italy, 573-578.
11. **Castellini, C.; Mourvaki, E.; Dal Bosco, A. and Galli, F. (2007):** Vitamin E biochemistry and function: A case study in male rabbit. Reprod. In Domestic Animal, 42:248-256.
12. **Castellini, C.; Dal Bosco, A. and Bernardini, M. (2001):** Improvement of lipid stability of rabbit meat by vitamin E and C administration. J. Sci. Food and Agric., 81:46-53.
13. **Castellini, C.; Dal Bosco, A.; Bernardini, M. and Cyril, H. (1998):** Effect of dietary vitamin E on the oxidative stability of raw and cooked rabbit meat. Meat Sci., 50:153-161.
14. **Chan, A. C. (1993):** Partners in defense, vitamin E and vitamin C. Can. J. Physiol. Pharmacol., 71:725-731.

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*2<sup>nd</sup> Int. Conf. on Vet. Med. (Moshtohor), Benha Univ*

15. Chew, B.P. (1995): Antioxidant vitamins affect food animal immunity and health. *J. Nutr.*, 125:1804-1808.
16. Corino, C.; LoFiego, D.; Macchioni, P.; Pastorelli, G.; Giancamillo, A.; Domeneghini, C. and Rossi, R. (2007): Effect of dietary conjugated linoleum acid and vitamin E on meat quality and adipose tissue in rabbits. *Meat Sci.*, 76:19-28.
17. Dal Bosco, A.; Castellini, C.; Bianchi, L. and Mugnai, C. (2004): Effect of dietary linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. *Meat Sci.*, 66:407-413.
18. Duncan, D. B. (1955): Multiple range and multiple-F-test, *Biometrics* 11:1-42.
19. El-rahim, M. I. A.; El-Gaafary, M.N.; Tawfeek, M.I.; El-Kelawy, H.M. and Rawia, S.A. (1995): Effect of dietary supplementation of different levels of zinc on growth performance, nutrient digestibility, mineral metabolism, blood constituents, organ histopathology and reproductive efficiency in NZW rabbits. *Egypt. J Rabbit Sci.*, 5(1): 11-31.
20. El-Husseiny, O.; Ghazalah, A.A.; Arafa, S.A.; Omar, N.E. and Manyalawy, M.A. (1997): Effect of vitamin A and E level and their interaction on the growth performance of growing rabbits. *Egypt. J. Rabbit Sci.*, 7(1): 13-25.
21. Fekete, S.G. and Kellems, R.O. (2007): Interrelationship of feeding with immunity and parasitic infection: a review. *Vet. Med. Czech Academy of Agric. Sci., Prague, Czech Republic*, 52(4): 131-143.
22. Ferreira, W.M.; Cavalcante, S.G.; Naranjo, A.P. and Santiago, G. S. (2002): Bioavailability of different zinc sources for rabbits. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia. Escola de Veterinaria, Universidad Federal de Minas Gerais, Belo Horizonte, Brazil*, 54(6): 636-642.
23. Gomeri, C. (1952): Calcium method for detection of B-lymphocytes. In theory and practice of histologic techniques 1977. Edited by Bancroft, J.D. and Stevens, A. S., Churchill living stone, Edinburgh, New York, pp. .
24. Hawks, P.B.; Oser, B.L. and S. nerson, W.H. (1954): Practical physiological chemistry. 13 Ed., the Mcgrow-Hill, Book Co., New York.
25. Hidiroglou, M.; Batra, T.R. and Ivan. (199): Effect of supplemental vitamin E and C on the immune responses of calves. *J. Dairy Sci.*, 78:1578-1583.
26. Lodja, Y.; Gassran, R. and Schieble, I.H. (1976): Enzyme histochemische methoden springen Verlag. Berlin, Heidelberg. Pp. 145-151.
27. Lukefahr, S.D.; Van Vleck, L.D. and Roberts, J.D. (1992): Estimates of components of variance and covariance of carcass traits in rabbits using animal model. *J Applied Rabbit Research*, 15:259-273.
28. Maraib, I.F.M.; Ayyat, M.S. and El-Monem, U.M.A. (2004): Heat stress and its limitation using integrated feed with zinc and magnesium, in growing male New Zealand White rabbits, in temperate and hot conditions in Egypt. *Rivista di Coniglicoltura. Gruppo Calderini Edagricole Srl, Bologna, Italy: 2004*, 41(3): 41-45.
29. Maynard, L.A. (1979): *Animal Nutrition*. 7<sup>th</sup> ed. McGraw-Hill Book Company, Inc. New York, Toronto, London.

30. **McCormic, B. and Green, H.L. (1994):** Vitamins. In: Burtis, C.A. and Ashwood, E.R. (Eds.), Tiers Textbook of Clinical Chemistry. 2<sup>nd</sup> Ed., Philadelphia, W.B., Sounder, pp. 1275-1316.
31. **Meshreky, S. and Shaheed, I. (2003):** Efficiency of vitamin E and selenium administration on growth performance, puberty and anatomical and histopathological traits of female genitalia in New Zealand White rabbits Egyptian J. Nutr. And Feeds, 6: 299-312.
32. **Niki, E. (1987):** Interaction of ascorbic acid and  $\alpha$ -tocopherol. Ann. NY Acad. Sci. 102: 186-199
33. **N.R.C. (1977):** Nutrient requirements of rabbits. National Academy of Science, Washington, D.C., USA.
34. **Oriani, G.; Corino, C.; Pastorelli, G.; Ritieni, A. and Salvatori, G. (2001):** Oxidative status of plasma and muscle in rabbits supplemented with dietary vitamin E. J. Nutr. Biochem., 12:138-143.
35. **Reed, D. (1992):** Interaction of vitamin E , ascorbic acid, and glutathione in protection against oxidative damage. In: Packer, L. and Fuchs, J. Editors, Vitamin E in health and disease, Marcel Dekker, New York, 269-281.
36. **SAS (1996):** Statistical Analysis System. Procedures Guide: Version 6.12 Edition. SAS Institute, INC., Cary, NC, USA.
37. **Sahin, K. and Kucuk, O. (2001):** Effects of vitamin C and vitamin E on performance, digestibility of nutrients and carcass characteristics of Japanese quills rearing under chronic heat stress. J. Anim. Physiol. Anim. Nutr., 85:335-341.
38. **Sallam, S.M.A.; Nasser, M.E.A.; Yousef, M.S.H.; El-Morsy, A.M.; Mahmoud, S.A.S. and Yousef, M.I. (2005):** Influence of aluminum chloride and ascorbic acid on performance, digestibility, and caecal microbial activity and biochemical parameters of rabbits. Res. J. of Agriculture and Biological Sci., 1 (1):10-16.
39. **Sedki, A.; Ismail, A.; Abou El-Ella, S.; Abou El-Wafa, S. and Abdallah, A. (2002):** Performance and immune function of growing rabbits as affected by vitamin C and E through the summer season. Egyptian J. Agric. Res , 80: 847-864
40. **Selim, N.A.; Abdel-Khalek, A.M.; Nada, S.A. and El-Medany, Sh.A. (2008):** Response of growing rabbits to dietary antioxidant vitamins E and C. 1. Effect on performance. In: Proc. 9<sup>th</sup> World Rabbit Congress- June 10-13, 2008, Verona – Italy, 803-807.
41. **Selim, N. A.; Soliman, A. and Abdel-Khalek, A. (2004):** Effect of drinking water temperatures and some dietary feed additives on performance of heat stressed rabbits. In: Proc. 8<sup>th</sup> World Rabbit Congress, 2004 September, Puebla, Mexico, 945-953.
42. **Shetaewi, M.M. (1998):** Efficacy of dietary high levels of antioxidant vitamins c and E for rabbits subjected to crowding stress. Egyptian J. Rabbit Sci., 8(2):95-112.
43. **Shindi, P.; Dass, R.S.; Garg, A.K.; Chaturvedi, V.K. and Kumar, R. (2006):** Effect of zinc supplementation from different sources on growth, nutrient digestibility, blood metabolic profile, and immune response of male guinea pigs. Biol. Trace Element Res., 112 (3):250-258
44. **Siegel, B.V. and Morton, J.I. (1977):** Vitamin C and the immune response. Experimentia, 15:393.

45. Swain, B.K.; Johri, T.S. and Majumdar, S. (2000): Effect of supplementation of vitamin E and selenium and their combination on the performance and immune response of broilers. *Br. Poultry Sci.*, 41(3):287-292.
46. Wen, J.; Lin, J. and Wang, H.M. (1997): Effect of dietary vitamin E and ascorbic acid on the growth and immune function of chicks. *Chinese Agric. Sci.*, 145-149.
47. Zahran, S.M.; Zeweil, H.S. and Ahmed, M.h. (1996): Effect of zinc bacitracin inclusion in diets with different dietary fiber levels on growth performance, digestibility, carcass and some blood constituents of growing rabbits. *Alexandria J. of Agric. Res.*, 41(2):93-110.

### **Legend of Figures**

- Fig. (1): Lymph node from rabbits of control group showing few B-cell reactions, which took the blackish discoloration. Alkaline phosphatase. Bar=100µm.
- Fig. (2): Lymph node from rabbits of group 5 showing increase in the number of activated B lymphocytes. Alkaline phosphatase. Bar=100µm.
- Fig. (3): Spleen from rabbits of control group showing B-cell reactions, which took the blackish discoloration. Alkaline phosphatase. Bar=100µm.
- Fig. (4): Spleen from rabbits of group 5 showing an increase in the number of activated B lymphocytes. Alkaline phosphatase. Bar=100µm and the insert (Bar=50 µm).
- Fig. (5): Lymph node from rabbits of control group showing few T-cell reactions, which took the brown color. Non specific esterase. Bar=50µm.
- Fig. (6): Lymph node from rabbits of group 5 showing an increase in the number of T-cells. Non specific esterase. Bar=50µm.

Table (1): Physical and chemical composition (%) of the experimental diet

Ingredients	Control basal diet
<b>Physical composition:</b>	
Yellow corn	29.0
Soybean meal	12.6
Wheat bran	10.0
Berseem hay	41.8
Molasses	5.0
Vegetable oil	-
Common salt	0.50
Limestone, ground	-
Di-calcium phosphate	0.55
Methionine	0.25
Premix*	0.30
<b>Total</b>	<b>100.0</b>
<b>Chemical composition:</b>	
Dry matter	88.83
Crude protein	16.42
DE (Kcal/kg diet)	2602
Crude fiber	13.82
Ether extract	3.03
Nitrogen free-extract	57.17
Calcium	0.74
Total phosphorous	0.49

\*Five star and Egavet premix: Each 3 kg contain: Vitamins: vit. A=12000000 IU, vit. D = 2500000IU, vit. E=10000 mg, vit. K<sub>3</sub>1000 mg, vit. B<sub>1</sub>=1000 mg, B<sub>2</sub>=5000 mg, B<sub>6</sub>=1500 mg, Niacin=30000 mg, biotin=50 mg, folic acid=1000 mg, pantothenic acid=10000 mg, Minerals: Manganese = 60000 mg, Zinc=50000 mg, Fe= 30000 mg, Copper = 5000 mg, Iodine= 500 mg, Selenium= 100 mg, Cobalt= 100 mg and Magnesium = 250000 mg. (carrier Ca CO<sub>3</sub> up to 3 kg).

**Table (2): Weekly feed intake (g/rabbit) of the different experimental groups**

Age in weeks	Groups				
	1	2	3	4	5
1 <sup>st</sup> week	434.0	390.0	409.0	410.0	406.0
2 <sup>nd</sup> week	494.0	464.0	482.0	496.0	436.0
3 <sup>rd</sup> week	516.0	566.0	560.0	555.0	514.0
4 <sup>th</sup> week	592.0	626.0	592.0	582.0	644.0
5 <sup>th</sup> week	712.0	726.0	736.0	710.0	746.0
6 <sup>th</sup> week	760.0	780.0	786.0	762.0	798.0
<b>Total intake 30-72 days</b>	<b>3508.0</b>	<b>3552.0</b>	<b>3565.0</b>	<b>3515.0</b>	<b>3544.0</b>

**Table (3): Body weight development of rabbits (g) in the different experimental groups**

Age in weeks	Groups				
	1	2	3	4	5
Initial weight	570.0±32.1	560.0±30.2	556.0±26.7	534.0±24.7	559.0±23.9
First week	768.0±29.4 <sup>b</sup>	816.0±25.4 <sup>a</sup>	775.0±25.3 <sup>b</sup>	760.0±22.6 <sup>b</sup>	756.0±27.2 <sup>b</sup>
second week	940.0±24.7 <sup>b</sup>	998.0±28.4 <sup>a</sup>	933.0±29.4 <sup>b</sup>	984.0±27.1 <sup>a</sup>	982.0±28.9 <sup>a</sup>
third week	1115±28.3 <sup>b</sup>	1239±30.7 <sup>a</sup>	1116±28.3 <sup>b</sup>	1123±31.7 <sup>b</sup>	1179±28.5 <sup>ab</sup>
fourth week	1271 ±30.1 <sup>b</sup>	1383±32.0 <sup>a</sup>	1294±26.2 <sup>b</sup>	1336±24.3 <sup>a</sup>	1355±28.5 <sup>a</sup>
fifth week	1499±32.9 <sup>b</sup>	1547±25.6 <sup>a</sup>	1564±23.9 <sup>a</sup>	1571±33.4 <sup>a</sup>	1581±37.0 <sup>a</sup>
6 <sup>th</sup> week	1666.0	1760.0	1751.0	1733.0	1785.0
(Final weight)	±36.1 <sup>b</sup>	±32.4 <sup>a</sup>	±33.8 <sup>a</sup>	±34.6 <sup>a</sup>	±35.3 <sup>a</sup>

\*Figures in the same row having the same superscripts are not significantly different (P<0.05).

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**Table (4): Rabbit performance and mortality of the five experimental groups**

Item	Groups				
	1	2	3	4	5
Number of rabbits	10	10	10	10	10
<b>Total feed intake (g) during whole period:</b>					
30-72 days	3508.0	3552.0	3565.0	3535.0	3544.0
<b>Total body weight gain (g):</b>					
30-72 days	1096.0	1200.0	1195.0	1199.0	1226.0
	±35.6 <sup>b</sup>	±31.5 <sup>a</sup>	±28.9 <sup>a</sup>	±32.6 <sup>a</sup>	±30.7 <sup>a</sup>
<b>Feed conversion:</b>					
30-72 days	3.20	2.96	2.98	2.93 <sup>3</sup>	2.89
Mortality %	20	-	-	-	-

\*Figures in the same row having the same superscripts are not significantly different (P<0).



**Table (5):** Carcass traits (%) of rabbits in the five experimental groups

Item	groups				
	1	2	3	4	5
No. of rabbits	3	3	3	3	3
Pre-slaughter weight (g)	1680±23.6 <sup>b</sup>	1800±36.4 <sup>a</sup>	1740±32.7 <sup>a</sup>	1710±27.1 <sup>a</sup>	1785±28.6 <sup>a</sup>
Dressed carcass weight (g)	944±27.2 <sup>b</sup>	1046±35.7 <sup>a</sup>	975±30.9 <sup>b</sup>	960±28.7 <sup>b</sup>	1050±26.9 <sup>a</sup>
Dressing (%)	56.19±0.95 <sup>b</sup>	58.11±0.89 <sup>a</sup>	56.03±0.92 <sup>b</sup>	56.15±1.07 <sup>b</sup>	58.82±0.94 <sup>a</sup>
For-limb	27.75±0.98	28.22±1.18	28.70±1.26	29.57±0.87	28.28±1.07
Hind-limb	39.79±1.16	39.41±2.02	40.90±1.62	38.70±1.28	39.21±1.36
Loin	21.47±0.68	21.49±0.59	19.96±0.84	20.86±0.66	20.18±0.75
Liver	8.32±0.36	8.06±0.61	7.76±0.48	8.56±0.54	7.89±0.68
Heart	0.51±0.06	0.49±0.08	0.53±0.05	0.55±0.07	0.50±0.09
Kidneys	0.85±0.15	0.87±0.13	0.83±0.16	1.03±0.13	0.88±0.14
Lung	1.05±0.12	1.09±0.09	1.10±0.13	1.07±0.17	1.11±0.15

\*Figures in the same row having the same superscripts are not significantly different (P<0.05).

Table (6): Nutrient digestibility and feeding values (%) of the experimental diet in the different treatments

Nutrients	groups				
	1	2	3	4	5
<b><u>Nutrient digest.</u></b>					
Dry matter	75.57±1.78	77.46±1.27	76.06±1.98	76.32±2.08	78.64±.63
Crude protein	80.15±1.46	82.34±1.62	81.15±1.83	81.96±2.11	82.87±2.05
Ether extract	86.55±1.89	87.18±2.13	86.74±1.92	87.08±1.55	87.11±1.72
Crude fiber	37.85±1.18	38.12±1.32	37.46±1.52	38.16±1.62	38.27±1.29
NFE	80.64±1.81	82.36±1.57	81.35±1.43	82.70 ±2.0	83.02±2.17
<b><u>Feeding values:</u></b>					
DCP	13.16±0.35	13.52±0.29	13.32±0.41	13.46±0.28	13.61±0.38
TDN	70.39±1.28	71.82±0.98	70.92±1.43	71.95±0.95	72.30±1.36

Table (7): Serum biochemical parameters (%) of rabbits in the five experimental groups

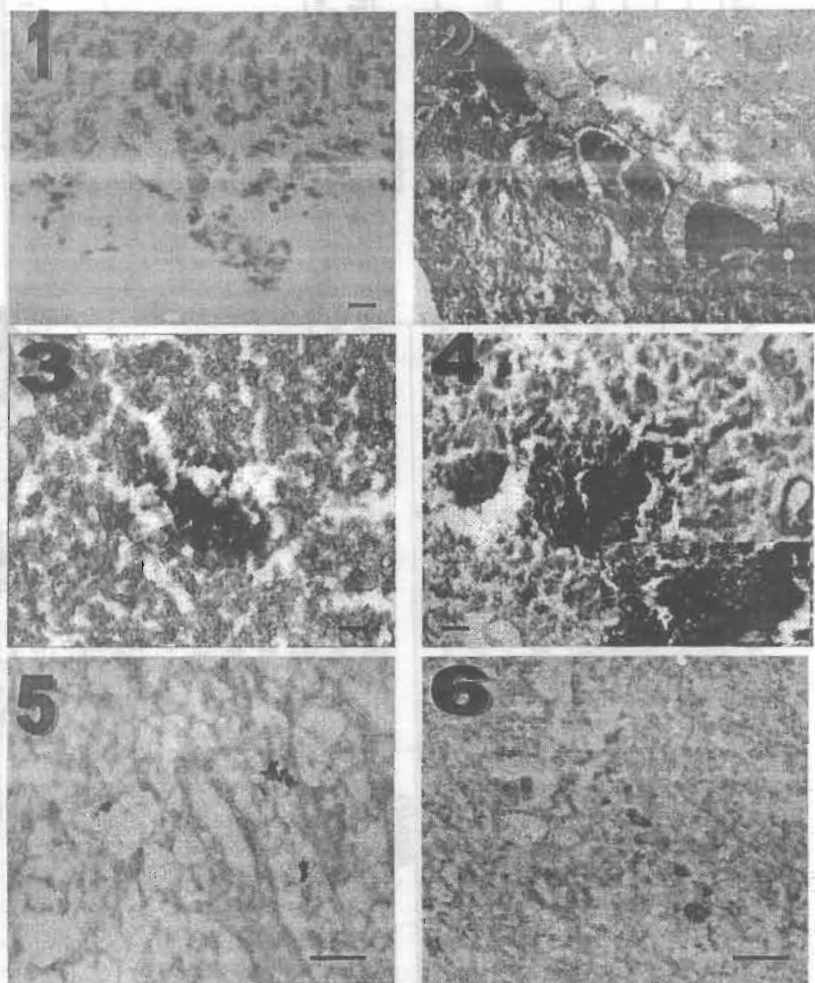
Biochemical parameters	groups				
	1	2	3	4	5
Total serum protein (g/dl)	7.5±0.98 <sup>b</sup>	8.2±0.86 <sup>b</sup>	7.9±0.78 <sup>b</sup>	9.9±0.86 <sup>a</sup>	9.4±0.78 <sup>a</sup>
Cholesterol (mg/dl)	185±7.8	194±9.3	188±8.4	1186±9.7	190±9.0
Urea (mg/dl)	10.6±0.94 <sup>b</sup>	10.9±0.11 <sup>b</sup>	11.0±0.78 <sup>b</sup>	14.8±1.3 <sup>a</sup>	13.6±0.9 <sup>a</sup>
α-tocopherol (µg/dl)	0.38±0.06 <sup>b</sup>	1.56±0.16 <sup>a</sup>	0.36±0.05 <sup>b</sup>	0.37±0.06 <sup>b</sup>	1.45±0.12 <sup>a</sup>
ascorbic acid (mg/dl)	1.44±0.14 <sup>b</sup>	1.39±0.18 <sup>b</sup>	2.95±0.31 <sup>a</sup>	1.42±0.1 <sup>b</sup>	2.56±0.28 <sup>a</sup>
zinc (µg/dl)	146.8±15.0 <sup>b</sup>	141.0±22.3 <sup>b</sup>	155.6±19.7 <sup>b</sup>	296.0±30.6 <sup>a</sup>	245.0±24.8 <sup>a</sup>

\*Figures in the same row having the same superscripts are not significantly different (P<0.05).

Table (8): Haematological picture (%) of rabbits in the five experimental groups

Blood parameter	Group				
	1	2	3	4	5
Total erythrocytes (×10 <sup>6</sup> µl)	6.13± 0.03 <sup>b</sup>	6.1 ± 0.09 <sup>b</sup>	5.9± 0.06 <sup>b</sup>	6.17± 0.09 <sup>b</sup>	6.47± 0.12 <sup>a</sup>
Total leukocytes (×10 <sup>3</sup> µl)	8.1± 0.05 <sup>b</sup>	8.07± 0.12 <sup>b</sup>	8.13± 0.09 <sup>b</sup>	7.93± 0.09 <sup>b</sup>	8.83±0.09 <sup>a</sup>
Neutrophiles (%)	28.3± 0.33 <sup>b</sup>	28.0± 0.57 <sup>b</sup>	28.0± 0.57 <sup>b</sup>	31.0± 0.88 <sup>a</sup>	32.6±0.33 <sup>a</sup>
Lymphocytes (%)	66.0 ± 0.57 <sup>c</sup>	67.6± 0.88 <sup>c</sup>	68.6± 0.3 <sup>b</sup>	66.6± 0.57 <sup>c</sup>	75.3±0.33 <sup>a</sup>
Monocytes (%)	3.0 ± 0.0	2.0± 0.0	4.0± 0.0	2.0 ± 0.0	3.0 ± 0.0
Eosinophiles (%)	2.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0
Basophiles (%)	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0

\*Figures in the same row having the same superscripts are not significantly different (P<0.05).



أثر إضافة مضادات الأكسدة و الزنك ميثونين على كفاءة الأداء و معاملات الهضم والاستجابة المناعية في الأرانب النامية تحت ظروف البيئة المصرية

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الملخص العربى

أجريت هذه التجربة لدراسة مدى تأثير إضافة كل من فيتامين هـ ، فيتامين ج ، الزنك ميثونين كل على حدة أو مجتمعة على كفاءة الأداء، خواص الذبيحة و معاملات هضم العليقة والاستجابة المناعية في أرانب النوزلندي النامية تحت ظروف البيئة المصرية. تم استخدام عدد ٥٠ من الأرانب النوزلندي النامية بعد الفطام (ذكور و إناث) في عمر ٣٠ يوم و متوسط وزن  $555 \pm 12$  جم، قسمت عشوائيا إلى خمس مجموعات متساوية بكل منها عدد ١٠ أرانب. أستمرت هذه التجربة لمدة ٤٢ يوم من الفطام حتى سن الذبح (٧٢ يوم) و اعتبرت الخمس الأيام الأخيرة من مدة التجربة فترة تجميعية. غذيت الأرانب في المجموعة الأولى على العليقة الضابطة الأساسية المحببة و التي تحتوي على الكميات الموصى بها من هذه الإضافات محل الدراسة. تم إضافة مستويين من كل من فيتامين هـ ، ج (٣٠٠ مجم ، ٤ جم/كجم عليقة أساسية) للأرانب في المجموعتين الثانية و الثالثة بالترتيب في حين غذيت الأرانب في المجموعة الرابعة على العليقة الضابطة الأساسية مضافا إليها ١٠٠ مجم من الزنك ميثونين/كجم عليقة. في المجموعة الخامسة أضيف خليط من فيتامين هـ ، ج ، الزنك ميثونين بمعدل (١٥٠ مجم ، ٢ جم ، ٥٠ مجم/كجم عليقة أساسية) بالترتيب. تم تكوين العليقة الضابطة الأساسية في صورة محببة و احتوت على المقررات الغذائية الموصى بها للأرانب النامية. أظهرت النتائج أن إضافة كل من فيتامين هـ ، ج ، الزنك ميثونين بمفرده أو في مخلوط يحسن معنويا الزيادة الكلية في وزن الجسم بمعدل ٩,٥ ، ٩,٠ ، ٩,٤ ، ١١,٩ % و كذلك يحسن معامل التحويل الغذائي بمعدل ٧,٥ ، ٦,٩ ، ٨,٤ ، ٩,٧ % في المجموعات المختبرة بالترتيب. لوحظ أن المعاملات الغذائية تؤثر على معدل التصافي حيث سجلت الأرانب في كل من المجموعة الثانية و الخامسة أعلى معدل للتصافي (٥٨,١١ and ٥٨,٨٢ %) بالمقارنة بالمجموعة الضابطة (٥٦,١٩ %) في حين لا

توجد فروق معنوية بين المجموعات المختبرة في الصفات الأخرى الذهبية. حدثت زيادة طفيفة في معاملات هضم كل المواد الغذائية وكذلك القيم الغذائية و سجلت المجموعة الخامسة أعلى القيم. كان التركيز الكلي للبروتين و اليوريا في مصل دم الأرانب المغذاة على الزنك في المجموعتين الرابعة و الخامسة أعلى ما يمكن بينما لم يكن هناك أي فروق معنوية بين المجموعات في تركيز الكوليسترول في الدم. وجد أن هناك زيادة معنوية في تركيز كل من فيتامين هـ ، ج ، الزنك في مصل دم الأرانب المغذاة على عليقة بها الإضافات مجتمعة. سجلت النتائج زيادة معنوية في العدد الكلي لكرات الدم الحمراء و البيضاء في المجموعة الخامسة المغذاة على الخليط. لوحظ أيضا زيادة معنوية في أعداد الخلايا الليمفاوية من نوعي B and T في كل من الغدد الليمفاوية و الطحال في كل المجموعات المختبرة و خاصة في المجموعة الخامسة التي غذيت على خليط من فيتامين هـ ، ج ، الزنك مثنونين. من هذه الدراسة نستخلص أن إضافة كل من فيتامين هـ ، فيتامين ج ، الزنك مثنونين بمفرده أو في خليط أعلى من الكميات الموصى بها في الجداول القياسية أدى إلى تحسن كفاءة للأداء و معاملات الهضم وكان للخليط أثر إيجابي واضح على الاستجابة المناعية في الأرانب التامية.