



Research article

Effects of Dietary Antioxidant Supplementation on Productive Performance, Egg Quality, Immune Response, and Antioxidant Status of Laying Hens

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ABSTRACT

This experiment was conducted to reveal the effects of dietary antioxidants on the productive performance, egg quality, immune response, and antioxidant status of laying hens. A total of 120, 45-week old Hy-Line Brown hens, were randomly allocated using a random number generator to four dietary treatments for 10 weeks: T1=control (basal diet), T2(PE300) =basal diet + 300 mg/kg of phytogetic extracts blend, T3(VE100) = basal diet + 100 mg/kg of vitamin E, and T4 (SP1)= basal diet + 1 g/kg of *Spirulina platensis*. Each treatment had 6 replicate cages with 5 hens per cage (n = 6 replicates/treatment); the cage was the experimental unit. Data were analyzed using a linear mixed-effects model with treatment, week, and treatment × week as fixed effects and cage within treatment as a random effect. All treatments improved laying rate (4.7–7.6% relative to control), egg mass, and the calculated feed conversion ratio compared to control ($P < 0.05$). The *Spirulina platensis* group had a significant increase in yolk color ($P < 0.01$). Serum IgY and IgA were increased by the dietary antioxidants, with a decrease in the pro-inflammatory cytokines, IL-1 β and TNF- α ($P < 0.05$). However, VE100 did not significantly increase IgA relative to the control. Increased serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities with decreased serum malondialdehyde (MDA) was shown in the treated hens ($P < 0.01$). The lower IL-1 β and TNF- α concentrations indicate a reduced pro-inflammatory status, not the presence of an inflammatory response. Data from this study suggest that dietary antioxidants given in the proper range improve productivity and immune response of laying hens.

1. Introduction

Oxidative stress negatively impacts poultry health and productivity. This is particularly the case for commercial laying hen operations due to exposure to many stressors. This includes high ambient temperature, pathogen exposure, and advanced age (Oke *et al.*, 2024). Oxidative stress occurs when there is an overproduction of reactive oxygen species, which exceeds the body's inbuilt defense system of antioxidants. This causes lipid membrane damage, protein damage, and suppression of the immune system (Surai *et al.*, 2019).

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This research indicates that the addition of dietary antioxidants can alleviate the damage caused by oxidative stress. Through meta-analysis, Darmawan *et al.* (2022) found that the use of phytogenic extracts increased egg production by 2.8% and improved the feed conversion ratio (FCR) by 0.15. Furthermore, Al-Rasheed *et al.* (2026) reported that *Spirulina platensis* (3 g/kg) improved the laying rate from 92.9% to 94.5% and improved antibody titers against Newcastle disease. In laying hens, the addition of Vitamin E improves antioxidant capacity and decreases malondialdehyde (MDA) (Zhao *et al.*, 2021; Liu *et al.*, 2019).

However, there is a deficiency of studies comparing different classes of antioxidants such as phytogenic extracts, microalgae, and vitamins in the same experimental setup. Therefore, the objectives of this study are to assess the effect of three classes of antioxidant supplements on the physiological performance of laying hens, investigate the effect on the immune response, and determine the antioxidant mechanisms involved. The Nrf2 and NF- κ B signaling pathways are proposed as possible mechanisms based on previous literature (Surai et al., 2021); however, these pathways were not measured in the present study

2. Materials and Methods

2.1 Experimental Design and Birds

The experiment was conducted at a poultry farm in Baqubah district, Diyala Governorate, from March 3, 2025 to May 11, 2025. Under controlled experimental conditions. All procedures were approved by the Animal Ethics Committee of Diyala University and complied with ARRIVE 2.0 guidelines. A total of 120 45-week-old Hy-Line Brown laying hens were used in this study. Hens were adapted for 14 days before the experimental period. During the adaptation period, hens were housed under the same environmental conditions as the experimental period and had ad libitum access to the basal diet and water. Initial health assessment included physical examination, body weight uniformity, and serological screening for common poultry pathogens. All hens received standard vaccination (Newcastle disease, infectious bronchitis, avian influenza) and were medicated with amprolium (0.0125%) for coccidiosis prophylaxis according to the farm's standard program.

Hens were randomly allocated using a random number generator (Microsoft Excel, 2019) to four treatment groups before the 10-week experimental period began. Each treatment had 6 replicate cages with 5 hens per cage ($n = 6$ replicates/treatment); the cage was the experimental unit. Mortality was recorded daily; no hens were excluded from the analysis.

2.2 Dietary Treatments

The four dietary treatments were as follows:

- T1 (Control): Basal diet without any antioxidant supplementation
- T2 (PE300): Basal diet + 300 mg/kg of a phytogenic extract blend
- T3 (VE100): Basal diet + 100 mg/kg of vitamin E (DL- α -tocopheryl acetate, purity $\geq 50\%$)
- T4 (SP1): Basal diet + 1 g/kg of *Spirulina platensis* powder

The phytogenic blend was obtained from Delacon Biotechnik GmbH, Steyregg, Austria (commercial product: Biostrong® 510), batch no. BSL-2024-0815, and contained carvacrol ($\geq 4.5\%$), cinnamaldehyde ($\geq 2.5\%$), and capsicum oleoresin ($\geq 1.0\%$) as active compounds, with a total active compound content of $\geq 8.0\%$ (w/w).

Vitamin E was obtained from DSM Nutritional Products Ltd., Kaiseraugst, Switzerland (commercial product: ROVIMIX® E-50), batch no. VE-2024-0387, in the form of DL- α -

tocopheryl acetate adsorbed on silica carrier, with a declared purity of $\geq 50\%$ (500 IU/g)

Spirulina platensis was obtained from Earthrise Nutritionals, LLC, Irvine, CA, USA (commercial product: Earthrise® Spirulina), batch no. SP-2024-1122, with the following analyzed composition: crude protein 58.5%, phycocyanin 10.2%, β -carotene 0.28%, total carotenoids 0.42%, crude fiber 6.8%, and ash 7.5% (on a dry matter basis). Diets were formulated according to Hy-Line Brown Commercial Management Guide (2023) recommendations. The ingredient composition and calculated and analyzed nutrient content of the basal diet are presented in Table 1.

Table 1. Ingredient composition and nutrient content of the basal diet (as-fed basis)

Ingredient	Percentage %
Corn	62.50
Soybean meal (44% CP)	24.00
Limestone	9.00
Dicalcium phosphate	2.00
Vegetable oil	1.50
Salt	0.30
DL-Methionine	0.15
L-Lysine	0.10
Vitamin-mineral premix ¹	0.25
Calculated nutrient content	
Metabolizable energy (kcal/kg)	2750
Crude protein (%)	16.50
Calcium (%)	3.80
Available phosphorus (%)	0.45
Lysine (%)	0.85
Methionine (%)	0.42
Analyzed nutrient content	
Crude protein (%)	16.42
Calcium (%)	3.75
Available phosphorus (%)	0.43
Background vitamin E (mg/kg)	12.5

¹Vitamin-mineral premix provided per kg of diet: vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin K3, 2 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; vitamin B12, 0.02 mg; niacin, 30 mg; pantothenic acid, 10 mg; folic acid, 0.5 mg; biotin, 0.1 mg; manganese, 80 mg; zinc, 60 mg; iron, 40 mg; copper, 8 mg; iodine, 0.5 mg; selenium, 0.2 mg.

Mortality data: No treatment-related mortality occurred during the 10-week experimental period.

2.3 Housing and Environmental Conditions

Hens were housed in wire cages (40 cm × 45 cm × 45 cm) in an environmentally controlled house. The environmental conditions were maintained as follows: temperature $22 \pm 2^{\circ}\text{C}$, relative humidity $55 \pm 5\%$, and a lighting program of 16-hour light:8-hour dark. Feed and water were provided *ad libitum* throughout the experimental period.

2.4 Blood Sample Collection and Analysis

At weeks 0, 5, and 10, blood samples were collected from the wing vein of 2 hens per cage. Blood samples were centrifuged first at $3,000 \times g$ for 15 min at 4°C , and serum was subsequently separated and stored at -20°C until analysis. For all blood parameters, values from individual hens within a cage were averaged to obtain one replicate value per cage before statistical analysis.

2.5 Laboratory Analyses

Serum immunoglobulin IgY concentrations were measured using the Chicken IgG ELISA Quantitation Set (Catalog No. E30-10, Bethyl Laboratories, Montgomery, TX, USA). The assay measures chicken IgY (not IgG-equivalent). The linear range of detection was between 25 and 200 mg IgY/mL. Intra- and inter-assay coefficients of variation were $< 8\%$ and $< 10\%$, respectively. Samples were diluted appropriately and measured in duplicate.

Serum IgA concentrations were measured using the Chicken IgA ELISA Kit (Catalog No. E33-103, Bethyl Laboratories, Montgomery, TX, USA). The assay range was 1.37–1000 ng/mL, with a sandwich ELISA format. Intra- and inter-assay coefficients of variation were $< 8\%$ and $< 10\%$, respectively. Samples were diluted appropriately and measured in duplicate.

Serum IL-1 β concentrations were measured using the Chicken Interleukin 1 Beta ELISA Kit (Catalog No. MBS703082, MyBioSource, San Diego, CA, USA). The detection range was 0.27–200 pg/mL, with a minimum detectable dose of less than 0.27 pg/mL. Intra- and inter-assay coefficients of variation were $< 8\%$ and $< 10\%$, respectively. Samples were diluted appropriately and measured in duplicate.

Serum TNF- α concentrations were measured using the Chicken Tumor Necrosis Factor Alpha ELISA Kit (Catalog No. MBS2509660, MyBioSource, San Diego, CA, USA). The detection range was 7.8–500 pg/mL, with a sensitivity of 3.1 pg/mL. Intra- and inter-assay coefficients of variation were $< 10\%$ and $< 12\%$, respectively. Samples were diluted appropriately and measured in duplicate.

Serum superoxide dismutase (SOD) activity was measured using the Superoxide Dismutase Assay Kit (Catalog No. 706002, Cayman Chemical, Ann Arbor, MI, USA). The assay measures all three types of SOD (Cu/Zn-, Mn-, and Fe-SOD) with a detection limit of 0.005 U/mL.

Serum glutathione peroxidase (GSH-Px) activity was measured using the Glutathione Peroxidase Assay Kit (Catalog No. 703102, Cayman Chemical, Ann Arbor, MI, USA). The assay measures GPx activity down to 50 U/mL using a coupled reaction with glutathione reductase.

Serum malondialdehyde (MDA) was measured using the TBARS Microplate Assay Kit (Catalog No. RDSM148, Reddot Biotech, Kelowna, BC, Canada). The detection range was 0.5–25 $\mu\text{mol/L}$.

2.6 Productive Performance and Egg Quality

Egg production was recorded daily, and egg weight was measured weekly. Egg mass was calculated as egg weight $\times$ laying rate. Feed intake was recorded weekly, and FCR was calculated as feed intake $\div$ egg mass. Egg mass and FCR were calculated separately for every experimental replicate before statistical analysis, not from final treatment means.

Egg quality parameters (yolk color, albumen height, Haugh unit, shell thickness, and shell weight) were measured at weeks 0, 5, and 10. Baseline (week 0) values were included to demonstrate baseline comparability among treatments.

2.6 Statistical Analysis

All data were analyzed using a linear mixed-effects model (SAS 9.4, SAS Institute, Cary, NC, USA) with the following model:

$$Y_{ijkl} = \mu + T_i + W_j + (T \times W)_{ij} + C(T)_{ik} + \epsilon_{ijkl}$$

Where:

- T_i = fixed effect of treatment ($i = 1-4$)
- W_j = fixed effect of week ($j = 0, 5, 10$)
- $(T \times W)_{ij}$ = treatment $\times$ week interaction
- $C(T)_{ik}$ = random effect of cage within treatment
- ϵ_{ijkl} = residual error

The covariance structure was compound symmetry (CS), selected based on AIC/BIC criteria. Assumption tests for normality (Shapiro-Wilk) and homogeneity (Levene's test) were confirmed ($P > 0.05$). Multiple comparisons were performed using Tukey's HSD test. Exact P-values, F-statistics, degrees of freedom, pooled SEM, and effect sizes are reported in all tables and figures. Planned contrasts were used for comparing the control with supplemented treatments and the three antioxidant classes. When period averages were analyzed, each replicate-level average was calculated by averaging weekly values for each cage across the 10-week period before one-way ANOVA. Sample size justification was based on power analysis ($\alpha = 0.05$, power = 0.80, expected effect size = 0.5).

3. Results

3.1 Productive Performance

The effects of dietary antioxidant supplementation on productive performance are presented in Figure 1. All supplemented treatments improved laying rate (4.7–7.6% relative to control), egg mass, and FCR compared to the control ($P < 0.05$). The VE100 laying-rate improvement was approximately 4.7% relative to the control. FCR decreased (improved) in all supplemented treatments. Egg weight and feed intake did not differ significantly among treatments ($P > 0.05$).

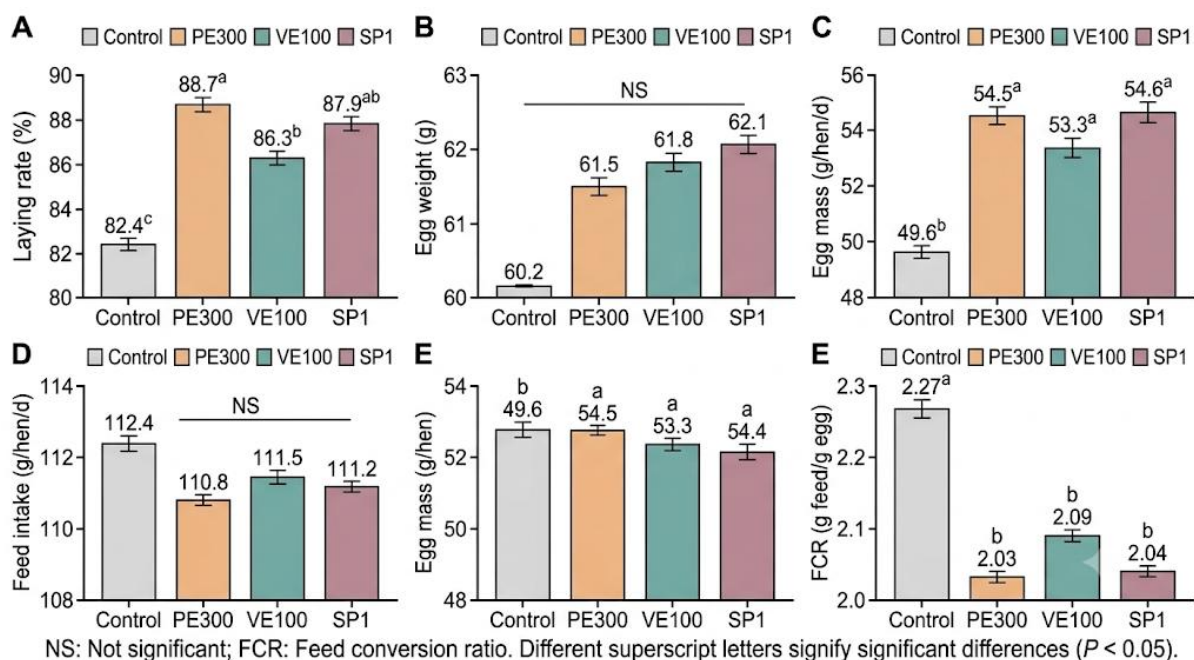


Figure 1. Effects of dietary antioxidants on productive performance of laying hens. Values are means of 6 replicate cages per treatment (5 hens/cage). Error bars represent pooled SEM. ^{a-c}Means with different superscript letters differ significantly ($P < 0.05$). NS: Not significant; FCR: Feed conversion ratio. Control = basal diet; PE300 = 300 mg/kg phytogetic extract blend; VE100 = 100 mg/kg vitamin E; SP1 = 1 g/kg *Spirulina platensis*.

3.2 Egg Quality

The effects of dietary antioxidant supplementation on egg quality are presented in Figure 2.

Table 2. Effects of dietary antioxidants on egg quality parameters of laying hens at 10.

Parameter	T1 (Control)	T2 (PE300)	T3 (VE100)	T4 (SP1)	P-value
Haugh unit	82.4 ± 1.1	83.7 ± 0.9	84.1 ± 1.0	83.2 ± 0.8	NS
Yolk color (Roche)	6.5 ± 0.3 ^b	7.2 ± 0.3 ^b	6.8 ± 0.2 ^b	9.8 ± 0.4 ^a	<0.01
Egg shell thickness (mm)	0.38 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.38 ± 0.01	NS
Albumen height (mm))	6.8 ± 0.2	7.0 ± 0.2	7.1 ± 0.2	6.9 ± 0.2	NS

¹Means within a row with different superscripts differ significantly ($P < 0.05$). NS: Not significant

The *Spirulina platensis* group had a significant increase in yolk color ($P < 0.01$). No significant differences were observed among treatments for albumen height, Haugh unit, shell thickness, or shell weight ($P > 0.05$)

3.3 Immune Response

The effects of dietary antioxidant supplementation on immune response are presented in Table 3. Serum IgY and IgA were increased by the dietary antioxidants, with a decrease in the pro-inflammatory cytokines IL-1 β and TNF- α ($P < 0.05$). However, VE100 was not significantly different from the control for IgA. The lower IL-1 β and TNF- α concentrations indicate a reduced pro-

inflammatory status, not the presence of an inflammatory response. SP1 may be described as numerically, but not significantly, superior when the supplemented treatments do not differ from one another.

Table 3. Effects of dietary antioxidant supplementation on serum immune parameters¹

Parameter	T1 (Control)	T2 (PE300)	T3 (VE100)	T4 (SP1)	Pooled SEM	P-value
IgY (mg/mL)	1250 ^b	1420 ^a	1380 ^a	1450 ^a	45	<0.001
IgA (mg/mL)	285 ^b	320 ^a	298 ^b	325 ^a	12	<0.001
IL-1 β (pg/mL)	45.2 ^a	38.5 ^b	39.8 ^b	37.2 ^b	1.8	<0.001
TNF- α (pg/mL)	62.5 ^a	54.2 ^b	55.8 ^b	52.8 ^b	2.1	<0.001

¹Values are means of 6 replicate cages per treatment (5 hens/cage). ^{ab}Means within a row with different superscripts differ significantly ($P < 0.05$).

3.4. Antioxidant Status

The effects of dietary antioxidant supplementation on antioxidant status are presented in Figure 2. Increased serum SOD and GSH-Px activities with decreased serum MDA were shown in the treated hens ($P < 0.01$).

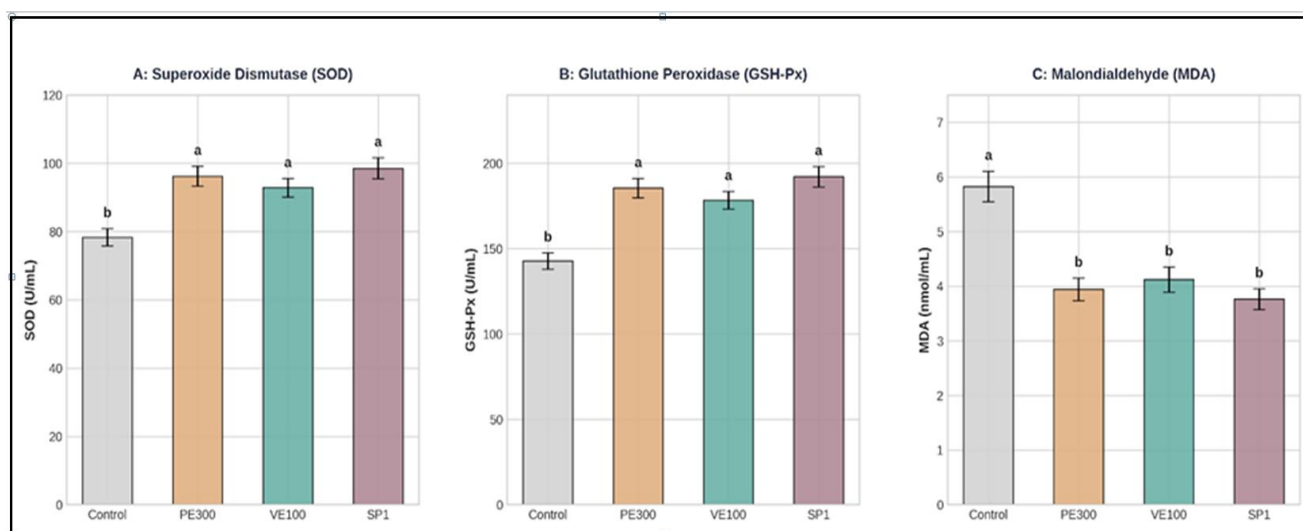


Figure 2. Effects of dietary antioxidants on serum antioxidant status and lipid peroxidation.

Values are means of 6 replicate cages per treatment (5 hens/cage). Error bars represent pooled SEM. ^{ab}Means with different superscript letters differ significantly ($P < 0.05$). SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; MDA: Malondialdehyde. Control = basal diet; PE300 = 300 mg/kg phytogenic extract blend; VE100 = 100 mg/kg vitamin E; SP1 = 1 g/kg *Spirulina platensis*.

4. Discussion

The current study demonstrated that dietary supplementation with a phytogenic extract blend, vitamin E, and *Spirulina platensis* improved productive performance, egg quality, immune response, and antioxidant status in laying hens. These findings are consistent with previous studies that have reported beneficial effects of dietary antioxidants in poultry (Darmawan et al., 2022; Oke et al., 2024; Song et al., 2026).

The improvement in laying rate and egg mass observed in the supplemented treatments may be attributed to the antioxidant properties of the tested additives, which can reduce oxidative stress and improve nutrient utilization (Surai et al., 2019). The decrease in FCR indicates improved feed efficiency in the supplemented groups. Similar improvements in productive performance have been reported with dietary phytogenic extracts (Darmawan et al., 2022; Anagnostopoulos & Mountzouris, 2025), vitamin E (Liu et al., 2019; Zhao et al., 2021), and *Spirulina platensis* (Al-Otaibi et al., 2022; Al-Rasheed et al., 2026).

The significant increase in yolk color in the *Spirulina platensis* group is likely due to the high content of carotenoids, particularly β -carotene, in *Spirulina platensis*. Carotenoids are known to be deposited in the yolk and enhance its color (Anderson et al., 1991; Zahroojian et al., 2011).

The increase in serum IgY and IgA concentrations in the supplemented treatments suggests enhanced humoral immune function. However, VE100 did not significantly increase IgA relative to the control, indicating that the effect of vitamin E on IgA may be less pronounced than that of the phytogenic blend or *Spirulina platensis*. The lower IL-1 β and TNF- α concentrations in the supplemented groups indicate a reduced pro-inflammatory status, which is consistent with the antioxidant and immunomodulatory properties of the tested additives (Surai et al., 2019; El-Ghareeb et al., 2023).

The increased SOD and GSH-Px activities and decreased MDA concentrations in the supplemented treatments indicate enhanced antioxidant status and reduced lipid peroxidation. These findings are consistent with previous studies that have reported improved antioxidant status in poultry supplemented with dietary antioxidants (Heng et al., 2021; Wang et al., 2025; Yu et al., 2025).

The Nrf2 and NF- κ B signaling pathways are proposed as possible literature-supported mechanisms for the observed effects, as these pathways were not measured in the present study (Surai et al., 2021). Nrf2 is a key transcription factor that regulates the expression of antioxidant enzymes, including SOD and GSH-Px (Surai et al., 2019). NF- κ B is a transcription factor that regulates the expression of pro-inflammatory cytokines, including IL-1 β and TNF- α (Surai et al., 2021). It is possible that the tested antioxidants activated Nrf2 and inhibited NF- κ B, leading to the observed improvements in antioxidant status and immune response. However, further studies are needed to confirm these mechanisms. It is important to note that only one dose of each non-equivalent additive was tested in this study. Therefore, no optimal supplementation range or superiority of one antioxidant class can be established. The SP1 treatment may be described as numerically, but not significantly, superior when the supplemented treatments do not differ from one another.

5. Conclusion

Dietary supplementation with a phytogenic extract blend, vitamin E, or *Spirulina platensis* improved productive performance, egg quality, immune response, and antioxidant status in laying hens. The VE100 laying-rate improvement was approximately 4.7% relative to the control. FCR decreased (improved) in all supplemented treatments. The lower IL-1 β and TNF- α concentrations indicate a reduced pro-inflammatory status. An improvement in the antioxidant status, was the result of increased SOD/GSH-Px and decreased MDA. Based on these findings, the addition of dietary antioxidants in the feed of laying hens is recommended as an effective nutritional decision in improving the productivity and the health of laying hens, especially those under the state of oxidative stress. Further studies are needed to determine the optimal supplementation range and to

elucidate the underlying mechanisms, including the potential involvement of the Nrf2 and NF- κ B pathways.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions: Taghreed Hadi, designed the experiment and wrote the manuscript. Manar Abdel Qader conducted the laboratory analyses and statistical analysis. and Fatima Mahmoud. managed the birds and collected the data. All authors read and approved the final manuscript.

References

- Abbas, A. O., Alaqil, A., El-Beltagi, H., Abd El-Atty, H. K., & Kamel, N. (2020). Modulating laying hens productivity and immune performance in response to oxidative stress induced by *E. coli* challenge using dietary propolis supplementation. *Antioxidants*, 9(9), 893, <https://doi.org/10.3390/antiox9090893>.
- Abo Ghanima, M. A., Elsadek, M., Taha, A., Abd El-Hack, M. A., Alagawany, M., Ahmed, B., ... & El-Sabrou, K. (2020). Effect of housing system and rosemary and cinnamon essential oils on layers performance, egg quality, haematological traits, blood chemistry, immunity, and antioxidant. *Animals*, 10(2), 245 ; <https://doi.org/10.3390/ani10020245>.
- Al-Khalaifah, H., Ibrahim, D., Kamel, A. S., Al-Nasser, A. Y., Abdelwarith, A. A., Roushdy, E. M., ... & Kishawy, A. T. (2024). Enhancing impact of dietary nano formulated quercetin on laying performance: egg quality, oxidative stability of stored eggs, intestinal immune and antioxidants related genes expression. *BMC Vet Res* 20, 494. <https://doi.org/10.1186/s12917-024-04327-x>
- Al-Otaibi, M. I. M., Abdellatif, H. A. E., Al-Huwail, A. K. A., Abbas, A. O., Mehaisen, G., & Moustafa, E.S (2022). Hypocholesterolemic, antioxidative, and anti-inflammatory effects of dietary *Spirulina platensis* supplementation on laying hens exposed to cyclic heat stress. *Animals*, 12(20), 2759. ; <https://doi.org/10.3390/ani12202759>.
- Al-Rasheed, M., El Sayed, M. S., Ramadan, K. M. A., Bendary, E., & Abdel-Raheem, S. (2026). The impact of *Spirulina platensis* supplementation on performance and immune response in laying hens. *Veterinary Research Communications*, 50,149,. <https://doi.org/10.1007/s11259-026-11069-8>.
- Anagnostopoulos, E. C., & Mountzouris, K. (2025). Gut detoxification and antioxidant responses in laying hens supplemented with phytogenics. *Journal of Applied Animal Nutrition*, 13(1), 1-13. <https://doi.org/10.1163/2049257x-20251009>.
- ANDERSON, D. W., CHUNG-SHIH, T., & ERNEST R.(1991). The Xanthophylls of *Spirulina* and Their Effect on Egg Yolk Pigmentation, *Poultry Science*, 70(1), 115–119. <https://doi.org/10.3382/ps.0700115>
- Darmawan, A., Hermana, W., Suci, D., Mutia, R., Sumiati, Jayanegara, A., & Ozturk, E. (2022). Dietary phytogenic extracts favorably influence productivity, egg quality, blood constituents, antioxidant immunological parameters of laying hens: A meta-analysis. *Animals*, 12(17), 2278; <https://doi.org/10.3390/ani12172278>.
- D'Alessandro, A., Di Luca, A., Desantis, S., & Martemucci, G. (2025). Antioxidant synergy in a mixture of powder plant leaves and effects on metabolic profile, oxidative status and intestinal morpho-histochemical features of laying hens. *Animals*, 15(3), 308 ; <https://doi.org/10.3390/ani15030308>
- El-Ghareeb, W., Kishawy, A. T., Anter, R. G. A., Gouda, A. A., Abdelaziz, W., Alhawas, B., ... & Ibrahim, D. (2023). Novel antioxidant insights of myricetin on the performance of broiler chickens and alleviating experimental infection with *Eimeria* spp.: Crosstalk between oxidative stress and inflammation. *Antioxidants*, 12(5), 1026.; <https://doi.org/10.3390/antiox12051026>.
- Fu, X., Liu, Y., Tong, L., Zhang, J., Zhuang, S., Gao, Y., & Zhang, J. (2026). Investigation on the regulatory effects of kaempferol on immune, inflammatory, and antioxidant functions in late laying hens. *Frontiers in Veterinary Science*, 13, (13),1763833. <https://doi.org/10.3389/fvets.2026.1763833>.

- Hartanto, S., Wardono, H., Kurnianto, H., Hantoro, F., Prasetyo, A., Haryanto, B., ... & Sugiharto, S. (2026). Dose-dependent effects of dietary quercetin on performance, egg quality, metabolic health, and antioxidant defense in laying hens: A systematic review and meta-analysis. *Veterinary world*, 19(1), 149–164. <https://doi.org/10.14202/vetworld.2026.149-164>
- Heng, N., Gao, S., Chen, Y., Wang, L., Li, Z., Guo, Y., ... & Qi, X. (2021). Dietary supplementation with natural astaxanthin from *Haematococcus pluvialis* improves antioxidant enzyme activity, free radical scavenging ability, and gene expression of antioxidant enzymes in laying hens. *Poultry science*, 100(5), 101045. <https://doi.org/10.1016/j.psj.2021.101045>
- Liu, Y., Zhao, L. H., Mosenthin, R., Zhang, J., Ji, C., & Ma, Q. (2019). Protective effect of vitamin E on laying performance, antioxidant capacity, and immunity in laying hens challenged with *Salmonella Enteritidis*. *Poultry Science*, 98(11), 5847–5854 <https://doi.org/10.3382/ps/pez227>.
- Madkour, M., Ali, S. I., Alagawany, M., El-Kholy, M. S., El-Baz, F., Alqhtani, A., ... & Elolimy, A. (2025). Dietary *Dunaliella salina* microalgae enriches eggs with carotenoids and long-chain omega-3 fatty acids, enhancing the antioxidant and immune responses in heat-stressed laying hens. *Frontiers in Veterinary Science*, 11, 1545433. <https://doi.org/10.3389/fvets.2025.1545433>.
- Obianwuna, U., Qiu, K., Chang, X., Zhang, H., Wang, J., Qi, G., ... & Wu, S. (2022). Enhancing egg production and quality by the supplementation of probiotic strains (*Clostridium* and *Brevibacillus*) via improved amino acid digestibility, intestinal health, immune response, and antioxidant activity. *Frontiers in Microbiology*, 13(13)987241. <https://doi.org/10.3389/fmicb.2022.987241>.
- Oke, O., Akosile, O., Oni, A., Opowoye, I. O., Ishola, C. A., Adebisi, J., ... & Abioja, M. (2024). Oxidative stress in poultry production. *Poultry science*, 103(9), 104003. <https://doi.org/10.1016/j.psj.2024.104003>.
- Song, W., Ma, J., Jia, C., Wang, Y., Yang, X., Han, L., Zhou, Z., & Yu, D. (2026). Dietary thyme essential oil supplementation improves production performance, egg quality, antioxidant status, and intestinal health in late-phase laying hens. *Poultry Science*, 105(2), 106774. <https://doi.org/10.1016/j.psj.2026.106774>.
- Surai, P. F., Kochish, I., Fisinin, V., & Kidd, M. (2019). Antioxidant defence systems and oxidative stress in poultry biology: An update. *Antioxidants*, 8(7), 235, <https://doi.org/10.3390/antiox8070235>.
- Wang, T., Chen, S., Lu, S., Li, H., Ni, M., Hao, J., ... & Qin, L. (2025). Effects of dietary selenium yeast supplementation on the production performance, egg quality, antioxidant and plasma biochemical parameters of laying hens. *Scientific Reports*, 15, 45104 (2025). <https://doi.org/10.1038/s41598-025-31644-4>
- Xu, H., Lu, Y., Li, D., Yan, C., Jiang, Y., Hu, Z., ... & Wang, Y. (2023). Probiotic mediated intestinal microbiota and improved performance, egg quality and ovarian immune function of laying hens at different laying stage. *Frontiers in Microbiology*, 14, 1041072. <https://doi.org/10.3389/fmicb.2023.1041072>.
- Yu, X., Li, J., Peng, R., Zhang, X., Yue, W., Wang, Y., Lan, Y., & Wang, Y. (2025). Flos *Lonicerae* and Baikal skullcap extracts improved laying performance of aged hens partly by modulating antioxidant capacity, immune function, cecal microbiota and ovarian metabolites. *Animals*, 15(19), 2882. <https://doi.org/10.3390/ani15192882>.
- Zahroojian, N., Moravej, H., & Shivazad, M. (2011). Comparison of marine algae (*Spirulina platensis*) and synthetic pigment in enhancing egg yolk colour of laying hens. *British poultry science*, 52(5), 584–588. <https://doi.org/10.1080/00071668.2011.610779>
- Zhao, H., Chen, Y., Wang, S., Wen, C., & Zhou, Y. (2021). Effects of dietary natural vitamin E supplementation on laying performance, egg quality, serum biochemical indices, tocopherol deposition and antioxidant capacity of laying hens. *Italian Journal of Animal Science*, 20(1), 2254–2262. <https://doi.org/10.1080/1828051X.2021.2002733>.
- Zhu, Y., Yin, L., Ge, J., Wu, X., Peng, Y., Zhang, T., & Jiang, M. (2020). Astaxanthin supplementation enriches productive performance, physiological and immunological responses in laying hens. *Animal Bioscience*, 34(3), 443–448. <https://doi.org/10.5713/ab.20.0550>.