

Effects of Dietary Purslane (*Portulaca oleracea* L.) Oil and Vitamin E Supplementation on Blood Biochemical Parameters and Oxidative Stress Biomarkers in Hydrogen Peroxide-Challenged Laying Hens

Hawraa Hassan Amin^{1*}

Ammar Qahtan-Shanoon²

1,2 Department of Animal Production, College of Agriculture, University of Kirkuk, Kirkuk, Iraq.

1 Email: akam24040@uokirkuk.edu.iq

2 Ammar.qahtan@uokirkuk.edu.iq

Abstract

Oxidative stress is one of the major factors adversely affecting the physiological status and health of laying hens. Therefore, this study was conducted to evaluate the effects of dietary supplementation with purslane (*Portulaca oleracea* L.) oil, either alone or in combination with vitamin E, on blood biochemical parameters and antioxidant status in laying hens subjected to hydrogen peroxide-induced oxidative stress.

A total of 160 Lohmann White laying hens were randomly allocated to eight experimental treatments with four replicates per treatment (five birds per replicate) for 90 days. Oxidative stress was induced by adding 0.5% hydrogen peroxide (H_2O_2) to the drinking water. Birds received purslane oil at 250 or 500 mL/ton of feed, either alone or in combination with vitamin E. At the end of the experiment, serum biochemical parameters, including glucose, total protein, cholesterol, and uric acid, together with antioxidant biomarkers, namely reduced glutathione (GSH) and malondialdehyde (MDA), were determined.

The results demonstrated that dietary supplementation with purslane oil, particularly when combined with vitamin E, significantly ($P \leq 0.05$) increased GSH concentration and decreased MDA concentration compared with the oxidative stress treatment, indicating an enhanced antioxidant defense system. Furthermore, supplementation maintained normal serum biochemical parameters without causing adverse effects on protein metabolism or the general physiological status of the birds.

In conclusion, dietary supplementation with purslane oil, especially in combination with vitamin E, effectively alleviated hydrogen peroxide-induced oxidative stress and enhanced the antioxidant status of laying hens. These findings suggest that purslane oil can be used as a natural dietary antioxidant to support physiological homeostasis and improve the health of laying hens under oxidative stress conditions.

Keywords: Laying hens; Purslane oil; Vitamin E; Oxidative stress; Hydrogen peroxide; Antioxidant status

Introduction

There are many stresses that modern production systems place on laying hens. One of the most significant is oxidative stress. It occurs when there are more free radicals and reactive oxygen species than the defense system has the capacity to counter. When there is oxidative stress, lipids, proteins and nucleic acids undergo damage that impairs the efficiency of many functions and the overall status of health of the birds [4,5].

There are both enzymatic and non-enzymatic antioxidants that birds have which, when combined, form an integrated antioxidant defense system. However, when prolonged stress occurs, this system can become compromised and there is a need for better and more natural nutritional strategies in order to enhance the defense system of the body. Because of their capacity to potentially help with the oxidative balance and damage, a lot of focus has occurred with the use of medicinal plants and plant-derived oils that have many bioactive compounds [6,3].

Purslane (*Portulaca oleracea* L.) has great potential as a source of bioactive compounds. It has many unsaturated fatty acids with appreciable amounts of α -linolenic acid, flavonoids, phenolic compounds, carotenoids, and both vitamins and minerals with antioxidant activity [8,6]. These constituents of purslane promote its positive physiological response, which includes reduced free radicals, increased antioxidant responses, and the preservation of cell membrane integrity and metabolism [3,9].

The use of purslane in the form of oil has additional significance. Purslane oil is a concentrated source of essential fatty acids and lipid-soluble bioactive compounds. It has great potential to improve the oxidative balance and the stress-related metabolic responses and physiological problems of birds. Growing evidence shows that the use of plant oils, especially those rich in unsaturated fatty acids, has positive effects in preventing oxidative injuries and improving cellular and tissue functions [6,8].

Vitamin E is a crucial lipid-soluble bioactive compound, and natural antioxidant that protects cellular membranes from lipid peroxidation. This vitamin also strengthens the antioxidant responses and lessens the effects of inflammation and oxidative stress. Therefore, it is in high demand in poultry diets [2]

The synergistic potential of purslane oil and vitamin E may be due to the presence of unsaturated fatty acids and antioxidant compounds in purslane oil and the biological activity of vitamin E. This combination may improve the antioxidant defense, less the impact of oxidative injury, and helps to preserve the physiological and biochemical balance in condition of oxidative stress [2].

Although several studies have investigated the beneficial effects of purslane and its derivatives in poultry nutrition, limited information is available regarding the combined effects of purslane oil and vitamin E on blood biochemical parameters and antioxidant status in laying hens exposed to hydrogen peroxide-induced oxidative stress. Therefore, it was hypothesized that dietary supplementation

with purslane oil, particularly in combination with vitamin E, could alleviate oxidative stress and improve the physiological and antioxidant status of laying hens. Accordingly, the present study was conducted to evaluate the effects of dietary purslane oil, alone or combined with vitamin E, on selected blood biochemical parameters and oxidative stress biomarkers in laying hens subjected

Material and Methods

One hundred sixty Lohmann White laying hens were used in the experiment which was carried out at the Poultry Research Farm, Department of Animal Production, College of Agriculture, University of Kirkuk. The aim of the study was to assess the consequences of the inclusion of purslane oil in the diet of laying hens and vitamin E on the physiological and biochemical consequences and on the oxidative stress caused by the use of hydrogen peroxide on the hens.

The study duration was 17 November 2025 to 15 February 2026 (90 Days). To study the effect of different experimental conditions, a total of 160 birds (20 in each treatment) were randomly assigned to the following groups: control, oxidative stress (0.5% H₂O₂ in drinking water), treatments supplemented with purslane oil (250 mL/ton, 500 mL/ton), and treatments supplemented with purslane oil and vitamin E. In addition to the previously mentioned groups, the following groups were included in the study: H₂O₂ + purslane oil (250, 500 mL/ton) and H₂O₂ + purslane oil + vitamin E.

During the experimental period, blood sampling was carried out for the evaluation of health status and oxidative stress, in

to hydrogen peroxide-induced oxidative stress [7,4].

addition to the assessment of the antioxidant status and comparison of the birds' responses to the different treatments.

Housing and Management

The laying hens were housed in a poultry facility of the Department of Animal Production. To optimize the rearing of the hens, aluminum cages arranged in tiers and equipped with feeders and automatic nipple drinkers were used. The hens were fed 105 g/day in two equal portions. This was done to promote daily feed and nutrient intake throughout the duration of the experiment. All treatments received drinking water ad libitum. For the oxidative stress treatments, hydrogen peroxide was added to the drinking water.

A 16 h light and 8 h dark schedule was used throughout this experiment. This schedule is used for laying hens to help maintain productive and physiological balance and is generally applied throughout the industry as standard management practice. As part of this schedule, the hens underwent a 14-day adaptation period prior to the application of the treatments to ensure they had time to adjust to the environment and to reduce the impact of environmental variability.

Experimental Materials

The subsequent treatments all utilized the same basal diet which was designed to be adequate for the hens throughout the entire experimental duration. This diet was comprised of yellow corn (43.90%), wheat (15.80%), and barley (4.00%) which provided the energy and supplemented with 22.30% of a soybean meal (48% crude protein) with the inclusion of 1.00% vegetable oil to raise the energy density of the diet.

To fulfill the hens' vitamin and mineral requirements, the diet included 2.50% premix, 0.45% dicalcium phosphate (DCP), and 9.20% calcium as limestone. Also included were 0.20% common salt, 0.25% choline chloride and 0.06% methionine, which was added to improve the diet's amino acid profile, since lysine was not limiting. The total sum of this diet was computed to 100%.

According to the computed chemical composition, the basal diet had metabolizable energy of 2724 kcal/kg, 16.97% crude protein, 1.502% lysine, 0.45% methionine, and 0.63% of methionine + cystine. It also included 4.505% Ca and 0.697% P, and the metabolizable energy to crude protein ratio was 160.532. These values confirmed that the hens' nutritional and physiological needs were satisfied by the experimental diet during the entire experimental period.

Statistical Analysis

The experiment was conducted using a Completely Randomized Design (CRD). Data were analyzed using the Statistical Analysis System (SAS) software (Version 9.4; SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was performed to evaluate the effects of the

Measured Traits

The studied traits were divided into two groups: hematological biochemical parameters and indicators of antioxidant status. The biochemical parameters were concentrations of serum glucose, total protein, uric acid, and cholesterol. Determination of concentrations of reduced glutathione (GSH) and malondialdehyde (MDA), which are considered major indicators of antioxidant status and of the oxidative stress of the organism, was used to evaluate the antioxidant status.

Blood samples of hens from all experimental treatments were taken at the end of the experiment. Serum was separated and used for routine laboratory analyses to evaluate the selected biochemical parameters and indicators of antioxidant status for assessing the impact of the varied treatments on the physiological status and the oxidative stress of laying hens.

experimental treatments. When significant differences among treatment means were detected, Duncan's Multiple Range Test was used for mean separation. Differences were considered statistically significant at $P \leq 0.05$. Results are presented as mean $\pm$ standard error of the mean (SEM).

Table (1). Effect of dietary supplementation with purslane oil and vitamin E as antioxidants against induced oxidative stress on blood biochemical parameters of laying hens (Mean $\pm$ Standard Error).

Treatment	Cholesterol (mg/dL)	Total protein(g/dL)	Glucose(mg/dL)	Uric acid(mg/dL)
T1	175.68 $\pm$ 4.2a	3.58 $\pm$ 0.17b	210.13 $\pm$ 1.2c	3.81 $\pm$ 0.18a
T2	165.28 $\pm$ 1.5a	3.50 $\pm$ 0.22b	210.23 $\pm$ 6.6c	3.59 $\pm$ 0.26b
T3	165.23 $\pm$ 2.9a	4.34 $\pm$ 0.22ab	226.22 $\pm$ 1.27b	3.55 $\pm$ 1.0a
T4	110.51 $\pm$ 3.4c	5.15 $\pm$ 0.28a	250.6 $\pm$ 1.5a	652. $\pm$ 0.19b
T5	$\pm$ 150.14 1.0b	4.55 $\pm$ 0.46a	230.130 $\pm$ 0.22b	3.30 $\pm$ 0.45ab
T6	$\pm$ 155.26 0.1 b	4.77 $\pm$ 0.22a	230.83 $\pm$ 5.8b	3.08 $\pm$ 0.09ab
T7	135.27 $\pm$ 0.6bc	4.40 $\pm$ 0.8a	235.35 $\pm$ 6 .8ab	3.70 $\pm$ 0.39ab
T8	128.16 $\pm$ 3.4c	4.63 $\pm$ 0.45a	235.130 $\pm$ 1.8ab	2.93 $\pm$ 0.17b

Note: Values are presented as mean $\pm$ SEM. Different superscript letters within the same column indicate significant differences among treatments at $P \leq 0.05$. NS = not significant

Antioxidant Status

The results presented in Table (2) revealed significant differences ($P \leq 0.05$) among the experimental treatments in oxidative stress biomarkers. Dietary supplementation with **purslane (*Portulaca oleracea* L.) oil**, either alone or in combination with vitamin E, significantly improved the antioxidant status of laying hens compared with those subjected to hydrogen peroxide-induced oxidative stress. This improvement was evidenced by a significant increase in reduced glutathione (GSH) concentration and a

significant decrease in malondialdehyde (MDA) concentration, indicating attenuation of oxidative stress and enhancement of the antioxidant defense system. These beneficial effects may be attributed to the presence of phenolic compounds and unsaturated fatty acids in purslane oil, which possess potent free radical-scavenging activity and effectively suppress the generation of reactive oxygen species (ROS). Furthermore, vitamin E, one of the most important lipid-soluble antioxidants, protects cellular membranes against lipid peroxidation [4,5].

Reduced glutathione (GSH) is one of the major endogenous antioxidants that plays a

crucial role in detoxifying hydrogen peroxide and scavenging free radicals, thereby maintaining intracellular redox homeostasis. Therefore, the elevated GSH concentration observed in the treatments supplemented with purslane oil and vitamin E indicates an enhancement of the endogenous antioxidant defense system and a reduction in oxidative cellular damage. These findings are consistent with those reported by [6,8], who demonstrated that phytogetic feed additives rich in phenolic compounds, together with vitamin E, enhance endogenous antioxidant activity and improve the resistance of poultry to oxidative stress.

Conversely, serum malondialdehyde (MDA) concentration was significantly lower in the treatments supplemented with purslane oil than in the oxidative stress treatment. Malondialdehyde is a well-established biomarker of lipid peroxidation and cellular membrane damage. Therefore,

its reduction indicates a lower rate of lipid peroxidation resulting from the inhibition of free radical formation and the enhancement of antioxidant defense mechanisms. Moreover, the combination of purslane oil and vitamin E produced a more pronounced effect than either supplement administered alone, suggesting a synergistic interaction between the natural antioxidants present in purslane oil and vitamin E in protecting cells against oxidative damage and improving the physiological status of laying hens [6,4].

Overall, these findings demonstrate that dietary supplementation with **purslane (*Portulaca oleracea* L.) oil**, particularly when combined with vitamin E, effectively enhanced antioxidant capacity and reduced oxidative stress biomarkers. Consequently, this supplementation improved the health status of laying hens exposed to oxidative stress, which is consistent with the findings reported by [5,6].

Table (2). Effect of dietary purslane oil and vitamin E supplementation on serum malondialdehyde (MDA) and reduced glutathione (GSH) concentrations in laying hens exposed to hydrogen peroxide-induced oxidative stress.

Treatment	MDA μmol/L	GSH μmol/L
T1	3.8 ± 0.10a	27.33± 0.48c
T2	4.6 ± 0.10a	21.77± 0.27c
T3	2.8 ± 0.17b	25.68± 2.0c
T4	1.7 ± 0.08c	38.66± 0.79a
T5	2.9 ± 0.18b	32.54± 2.04bc
T6	2.1± 0.24b	33.83± 0.66b
T7	2.1± 0.43a	35.86± 0.29a
T8	2.3± 0.11b	37.63± 0.39a

Note: Values are presented as mean $\pm$ SEM. Different superscript letters within the same column indicate significant differences among treatments at $P \leq 0.05$. NS = not significant

Discussion

The results of the present study showed that dietary supplementation with purslane oil and vitamin E in laying hens exposed to hydrogen peroxide-induced oxidative stress did not significantly affect some blood biochemical parameters, such as total protein, whereas differences were observed in other parameters, including glucose and uric acid, together with a numerical reduction in cholesterol concentration in some treatments. These findings suggest that the physiological response of birds to oxidative stress depends on the severity of stress, the type of antioxidant used, and its ability to maintain metabolic homeostasis within the body.

The absence of significant changes in total protein concentration may indicate that the experimental treatments did not adversely affect liver function or protein synthesis processes. It also reflects the ability of birds to maintain fluid balance and blood constituents despite exposure to oxidative stress. Previous studies have indicated that natural antioxidants and vitamin E contribute to the protection of cells and tissues against oxidative damage, thereby helping to maintain biochemical parameters within their normal physiological ranges. The numerical reduction in cholesterol concentration observed in some purslane oil treatments may be attributed to the presence of unsaturated fatty acids, phenolic compounds, and flavonoids in the plant, which contribute to improving lipid metabolism and reducing lipid peroxidation. In addition, vitamin E plays an important role in protecting membrane lipids and lipoproteins from oxidative

damage, thereby exerting a positive effect on lipid homeostasis. Studies conducted on laying hens have demonstrated that supplementation with natural antioxidants or vitamin E may reduce cholesterol concentrations and improve the oxidative status of birds.

Regarding glucose concentration, its reduction in the oxidative stress treatment compared with some purslane oil treatments may be associated with increased energy and nutrient utilization to cope with stress resulting from enhanced free radical production. Oxidative stress disrupts metabolic processes and increases energy requirements for the activation of antioxidant defense systems and the repair of damaged tissues. Supplementation with purslane oil and vitamin E may have improved physiological responses and reduced the adverse effects of oxidative stress, thereby helping to maintain blood glucose levels within normal ranges.

The relative increase in uric acid concentration observed in some treatments may be related to enhanced metabolic activity or antioxidant defense mechanisms. Uric acid is considered one of the antioxidant compounds in birds and contributes to the neutralization of free radicals. Furthermore, the fact that its values remained within the normal physiological range indicates the absence of pathological disturbances or adverse effects on protein metabolism.

Overall, the findings indicate that purslane oil and vitamin E contributed to alleviating the harmful effects of oxidative stress and maintaining the biochemical and physiological stability of laying hens. These effects may be attributed to the synergistic action between the antioxidant compounds present in purslane oil and the antioxidant activity of vitamin E in protecting cellular membranes, reducing lipid peroxidation, and improving the

efficiency of various metabolic processes.

Recommendations

1. The use of purslane oil and vitamin E as natural dietary supplements is recommended to help alleviate the adverse effects of oxidative stress in laying hens.
2. Further studies are recommended to evaluate the effects of purslane oil on additional physiological and immunological parameters.
3. Different levels of purslane oil and vitamin E should be investigated to determine the optimal supplementation level that achieves the best physiological response.
4. Further research is recommended to expand the investigation of the effects of purslane oil on antioxidant indicators and oxidative stress-related enzymes.

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