

Effects of using Purslane Oil on Antioxidant status and Biochemical traits of Laying Hens under Oxidative Stress Compared with Butylated Hydroxytoluene (BHT)

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Abstract

The objective of the research was to determine the effect of purslane oil in comparison to the synthetic antioxidant butylated hydroxytoluene (BHT) in the diets of laying hens under oxidative stress due to hydrogen peroxide, blood biochemical parameters of choice and the antioxidant status. The distribution was of 160 Lohmann White laying hens in five replicates and four hens in each replica randomly in eight dietary treatments in a completely randomized arrangement. These treatments included a control group, oxidative stress group, two doses of purslane oil in the diet and a mix of the two doses of purslane oil with hydrogen peroxide and BHT. The findings indicated that dietary treatments had significant effects. It was found that there were positive changes in the blood biochemical parameters and antioxidant status particularly the growing amount of glutathione concentration and the declining amount of cholesterol in the more efficient treatment. The best response was observed in most of the characteristics examined with the treatment having the highest concentration of purslane oil, with the next best result with most of the other oil supplement treatments, but the worst result on most indicators was found with the treatment using the oxidative stress alone. To sum up, purslane oil has a potential as a promising natural feed additive in relation to the reduction of the adverse effect of oxidative stress and the positive impact on the physiological activity of laying hens.

Keywords: laying hens, oxidative stress, hydrogen peroxide, antioxidant status.

Introduction

The poultry industry is considered one of the most essential industries and serves as an important source of high-quality animal protein. Laying hens are regarded among the least

expensive sectors within poultry production. Egg production is greatly influenced by the birds' genetic capacity, management conditions, as well as their physiological and nutritional status. Oxidative stress is recognized as one

of the most widely discussed subjects in scientific research because of its possible negative effects on laying performance and bird health through its harmful impact on metabolism, cellular integrity, and productive efficiency. When reactive oxygen species exceed the capability of the antioxidant defense system, oxidative stress occurs, leading to cellular damage and consequently causing adverse effects on normal physiological functions [1], [2]. Oxidative stress can lead to a reduction in egg production, impairment of egg quality, and negatively affect antioxidant defense mechanisms in laying hens, and impaired nutrient utilization, in addition to disrupting the normal biochemical and antioxidant status of laying hens. It may also induce lipid peroxidation and depletion of key antioxidant systems such as glutathione, ultimately compromising the bird's ability to maintain physiological homeostasis during the production period [1], [3]. These harmful effects highlight the importance of reducing oxidative stress in order to maintain both the productivity and health of poultry systems. In recent years, nutritional strategies have increasingly focused on the use of antioxidants to minimize oxidative stress in poultry. Among the commonly used antioxidants butylated hydroxytoluene (BHT) is a synthetic antioxidant which has been widely incorporated into animal diets to

prevent or delay oxidative damage and to improve the stability and quality of feed. Consequently, considerable attention has been directed toward evaluating the safety and effectiveness of BHT in animal production systems [4]. Therefore, some have resorted to adding medicinal plants that possess many therapeutic and medicinal properties in addition to their antioxidant action, as one plant contains many active substances that work together and in a synergistic manner to treat diseases [5,6] Among these plants is purslane (*Portulaca oleracea* L.) a herbaceous plant widely distributed throughout the world. Previous studies have reported its biological activities, including antioxidant, antimicrobial and anti-inflammatory, respiratory disease, and its beneficial effects on the kidneys, nervous system, and liver [7,8].

The action of the food antioxidants on the laying hens, in its turn, is the question that should be explored with the assistance of the concomitant analysis of the biochemical variables and antioxidant condition of the blood.

On this basis, the research was planned to elicit an argument on the effect of purslane oil and that of BHT, on laying hens, which were subjected to an oxidative stress of hydrogen peroxide. The research points were expected to determine the biochemical parameters and antioxidant status of the blood and

discuss the opportunities of purslane oil as a natural antioxidant feed additive to the layers feed.

Material and Methods

Birds, arrangement and position of the experiment.

This was carried out in Poultry Research Farm, Department of Animal production, College of Agriculture, University of Kirkuk where there were 160 Lohmann White laying hens. The objective of the experiment was to compare the effects of dietary purslane oil and synthetic antioxidant butylated hydroxytoluene (BHT) on laying hens subjected to induced oxidative stress, the presence of hydrogen peroxide and the resulting effects on selected biochemical parameters and antioxidant status. The experimental period was 91 days and 11 days of adaptation. The eight dietary treatments with 5 replicates each were randomly allocated to the birds with four hens each. The experiment was conducted using a completely randomized design.

Studied traits:

Individual and combined effect of purslane oil, BHT and hydrogen peroxide were tested using eight experimental treatments. The treatments were designed in the following way T1, basal diet,

(Control treatment) no supplement, T2, basal diet, 0.5% of hydrogen peroxide, T 3, basal diet, 250 mL/ton of purslane oil, T4, basal diet, 500 mL/ton of purslane oil, T5, basal diet, 0.5% of hydrogen peroxide+ Purslane oil (250 ml/ton), T6 basal diet, 0.5% of of hydrogen peroxide+ Purslane oil (500 ml/ton),T7 basal diet, 0.5% of of hydrogen peroxide+ Purslane oil (250 ml/ton) + BHT according to standard company recommendations,T8,basal diet, 0.5% of of hydrogen peroxide+ Purslane oil (500 ml/ton) + BHT according to standard company recommendations.

Management and feeding

Purslane oil was purchased from a local oil press, in addition to hydrogen peroxide (H₂O₂) at a concentration of 3.875%. The materials were gradually and partially mixed with the feed until complete homogeneity of the diet was achieved within each treatment. The hens were confined in four tier vertically packed aluminum cages in poultry house having plastic feeders and drinkers. The production guide was used to feed the hens at a rate of 105 g/hen/day and divided into two feeds of 07:00 h and 16:00 h. Mass of feed residues was measured and recorded using an electronic balance of 0.5 g sensitivity at the end of each week. It was observed that. The experiment was divided into six stages of production each stage was

14 days to see the variation of the parameters being studied with time.

Basal diet composition

The nutrient requirement was the basis on which the laying hen diet was formulated based on the recommendation of National Research Council [10]. The basal diet consisted of wheat (15.80%), corn (43.90%), oil (1.00%), barley (4.00%), soybean meal 48% (22.30%), laymix (2.50%), methionine (0.06%), dicalcium phosphate (0.45%), limestone (9.5%), salt (0.20%), and choline chloride (0.25%). This diet was estimated to have 2724 kcal/kg metabolizable energy, 16.97% crude protein, 1.502% lysine, 0.45% methionine, 0.63% methionine + cystine, 4.505% calcium and 0.697% phosphorus [10].

Measured traits

The research had two groups of research measured variables that comprised blood biochemical parameters and antioxidant status. The biochemical parameters of blood were glucose, total protein, uric acid and cholesterol. The measure of antioxidant status was taken to be the glutathione (GSH) and malondialdehyde (MDA). Blood samples were taken at the completion of the experiment in order to find out the selected serum biochemical parameters and indicators of antioxidants. The tests done on the biochemical

characteristics were total protein, uric acid, glucose, cholesterol and the measure of antioxidant status,

Statistical analysis

The analysis of the data was done according to absolutely randomized design through the use of SAS software (2005). Differences between the treatment means of level of 0.05 probability were significantly determined with the assistance of Duncan multiple range test.

Results

The situation of the present experiment revealed that the, blood biochemical parameters, and antioxidant status of Lohmann White laying hens. Overall, the tendency in the answers showed that the purslane oil-enriched groups and especially the enhanced amount of supplementation had a better response in physiological characteristics relative to the treatment of oxidative stress. With the treatment coding matching the research plan, T4, T3 and T6 often had the most positive overall responses, and T2 often had the worst values of most characteristics.

Blood biochemical parameters

The biomedical parameters of the blood reacted differently to the diet treatments. there were significant differences among treatments in Total Protein, where T4 showed a significant improvement compared

with the stress treatment. Treatment was influential on the uric acid concentration. The highest value was recorded in T4(3.37 ± 0.17), whereas the lowest value was found in T2 (1.49 ± 0.24).

The cholesterol level was greatly reduced in some of the treatments. The lowest cholesterol level was recorded in T4 (10.58 ± 0.2), followed by T3 (25.18 ± 0.9) and T6 (29.54 ± 0.4), whereas the highest levels were observed in T2 (77.57 ± 1.0) and T1 (74.14 ± 3.5). The tendency suggests that the groups which had more successful purslane oil intervention have better biochemical reaction.

Oxidative stress and antioxidant condition.

The diet therapies had a influence on the level of glutathione and malondialdehyde. For glutathione, the highest value was recorded in T4 (979 ± 0.69), followed by T3 (835 ± 1.98) and T6 (827 ± 0.68), whereas

the lowest values were found in T2 (306 ± 0.23) and T1 (326 ± 0.37).

The malondialdehyde values were also found to differ between treatments. The highest values were reported in T2 (105.9 ± 0.09), T1 (83.6 ± 2.00) whereas the lower values were observed in T4 (22.6 ± 0.09). Lastly, it was demonstrated that T4 was more productive in the, part of the blood biochemical indices, and the glutathione level, and T3 and T6 did not provide negative results on most of the variables. T2 on the other hand was more likely to have the lowest, or one of the lowest, values on most of the measured characteristics. On the whole, the pattern of response justifies the finding that purslane oil in the dietary supplement enhanced the physiological condition of laying hens in the circumstances of this experiment.

Table 1 Effect of dietary treatments on blood biochemical parameters of Lohmann White laying hens

Treatment	Total protein(g/dl)	Uric acid(mg/dl)	Glucose(mg/dl)	Cholesterol(mg/dl)
T1	3.55 ± 0.17 c	1.79 ± 0.16 ab	76.32 ± 3.13 ab	74.14 ± 3.5 a
T2	2.50 ± 0.21 c	1.49 ± 0.24 b	65.51 ± 3.5 b	77.57 ± 1.0 a
T3	6.63 ± 0.32 b	2.85 ± 1.26 ab	143.33 ± 1.7 ab	25.18 ± 0.9 ab

Treatment	Total protein(g/dl)	Uric acid(mg/dl)	Glucose(mg/dl)	Cholesterol(mg/dl)
T4	8.13 ± 0.38 a	3.37 ± 0.17 a	166.88 ± 1.5 a	10.58 ± 0.2 b
T5	6.00 ± 0.55 b	1.98 ± 0.35 ab	111.33 ± 5.4 ab	40.98 ± 1.0 ab
T6	6.07 ± 0.32 b	2.07 ± 0.08 ab	125.62 ± 6.3 ab	29.54 ± 0.4 ab
T7	4.60 ± 0.72 b	1.80 ± 0.29 ab	99.80 ± 4.8 ab	62.39 ± 0.6 ab
T8	5.77 ± 0.74 b	1.93 ± 0.17 ab	106.54 ± 1.8 ab	46.11 ± 2.4 ab

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

Table 2. Effect of dietary treatments on oxidative stress and antioxidant status of Lohmann White laying hens

Treatment	MDA (Mol/L μ)	GSH (Mol/L μ)
T1	83.6 ± 2.00 a	326 ± 0.37 c
T2	105.9 ± 0.09 a	306 ± 0.23 c
T3	22.9 ± 0.13 b	835 ± 1.98 ab
T4	22.6 ± 0.09 b	979 ± 0.69 a
T5	26.0 ± 0.16 b	617 ± 2.04 bc
T6	23.1 ± 0.23 b	827 ± 0.68 ab
T7	73.1 ± 2.44 a	350 ± 0.27 c
T8	26.3 ± 0.13 b	363 ± 0.34 c

Note: Values are presented as mean ± SEM. Different superscript letters within the same column indicate significant differences among treatments at $P \leq 0.05$.

Discussion

The available evidence has shown that purlane oil was a desirable impact in the blood biochemical traits and antioxidant condition in

Lohmann White laying hens that were subjected to the hydrogen peroxide-induced oxidative stress. The definition is in line with the existing literature where oxidative stress is identified as a reason behind the loss of metabolic homeostasis, down-regulation of productive characteristics and reduction of egg quality in chicken and dietary supplementation can be applied to stabilize redox balance, cellular integrity and nutrient feeding using antioxidant supplements [11], [12]. This interpretation is also proven by the blood biochemical results. In spite of the fact that the overall protein did not indicate significantly different results across the treatment, the decreased cholesterol of the better functioning groups and the more desirable values of glucose and uric acid indicate that purslane oil could have played a role in influencing the metabolism of the nutrients and regulating the lipids in the state of oxidative stress. This is in accordance with the well-established biological effects of bioactive compounds of plant origin that have been implicated in other animal and human studies to be metabolically regulated and hypolipidemic [13], [14]. The productive results are also supported by the antioxidant results especially the increase in the glutathione in the more desirable treatments meaning that the endogenous antioxidant

defense capacity is increased. Glutathione which is one of the important extracellular antioxidants of neutralizing reactive oxygen species and redox homeostasis has been shown to increase normally as a receptor of improved antioxidant defense [11], [12]. The obtained results put together indicate that purslane oil has the potential to be one of the possible naturally occurring antioxidant feed additives in the laying hens exposed to the oxidative stress and that the beneficial impact of the substance is most likely to be contingentized by the redox conditions improvement, the alleviation of stress-induced metabolic imbalance and the maintenance of egg formation in the hens. The fact which also proves this interpretation is that the plant bioactive constituents could have prominent metabolic and hypolipidemic effects [15]. In that way, the high decrease in cholesterol and the increase in glutathione that were established in the present paper may be considered to have biological merit and may be related to the increased metabolic rate during purslane oil supplementation.

Conclusion

In short, dieting purslane oil enhanced, the biochemical parameters in blood, and the antioxidant status of the Lohmann White laying hens which were subject to oxidative stress using hydrogen peroxide. The T4 was found to react well in respect of majority of the experimental characteristics with T3 and T6 reacting well in a few characteristics, reduced cholesterol, and

high glutathione. On the other hand, the only treatment of the oxidative stress (T2). In this connection, purslane oil can be regarded as the possible natural antioxidant nutritional supplement to overcome the negative effect of oxidative stress and promote the physiological performance of laying hens.

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