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Article

Physiological Performance of Laying Hens Undergoing Heat Stress and Fed Diets Containing Different Levels of Crude Artichoke *(Cynara Carduncellus)* and Mulberry *(Morus alba L.)* Leaf Powders, and Their Synergistic Mixture

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Abstract: Adding medicinal plants to poultry diets is typically aimed at enhancing the birds' health and physiological state, in addition to disease resistance. In this study, 144 Lohmann Brown laying hens at 23 weeks of age were randomly divided into six treatments (24 hens/treatment, with three replicates of eight hens/replicate). The six treatment groups were fed a standard diet supplemented with artichoke leaf powder, mulberry leaf powder, and a mixture of both, at the following ratios: 0, 1, 1.5, 1, 1.5, and 1.5+1.5 g/kg feed for each treatment, respectively. The following parameters were studied: cholesterol concentration, protein concentration, glucose concentration, calcium concentration, and phosphorus concentration. Blood was collected from the birds in each replicate every two weeks throughout the four-month study period, and the final average was calculated for each month. The results indicate that the synergistic mixture had a highly significant effect in improving the blood's biochemical parameters, including cholesterol concentration, total protein concentration, glucose concentration, and calcium and phosphorus levels. It can be concluded from this study that the addition of raw artichoke leaf powder, mulberry leaf powder, and their mixture to the diets of laying hens led to a significant improvement in the overall physiological and cellular characteristics of the blood. Consequently, this supplement can be used in laying hen diets to improve their physiological performance.

Keywords: Laying Hens, Heat Stress, Crude Artichoke, Mulberry Leaf, Synergistic Mixture

Introduction

Elevated environmental temperatures above normal conditions, particularly in broiler and laying hen breeding farms, lead to numerous nutritional challenges. Primarily, these include reduced feed intake, decreased growth rate, deteriorating health and immune status of the birds, and physiological stress. This physiological stress is responsible for a decline in poultry immunity and productivity [1], [2]. Consequently, recent research has focused on elucidating the mechanisms of heat stress and analyzing its reactive pathways within the body. This pursuit aims to understand the operational mechanics and mitigate the potential adverse effects on the birds.

Various approaches have been adopted to address these challenges, including diverse genetic treatments and interventions related to building design, insulation materials, and ventilation systems [3], [4]. Nutritional treatments have also been explored, involving the control of dietary components, particularly protein, energy, amino acid levels, and mineral elements [5]. Furthermore, plant-based feed additives have played a crucial role in reducing the severity of heat stress, exemplified by the use of Cordia myxa fruit [6].

One of the medicinally significant plants with widespread therapeutic use is the artichoke, scientifically known as Cynara carduncellus, commonly referred to as Artichoke. This plant belongs to the Asteraceae family, which includes several notable species such as Camomile (Matricaria chamomilla), Artemisia, Calendula, and Artichoke itself. Artichoke is rich in bioactive compounds including caffeic acid, chlorogenic acid, and cynarin—a pharmacologically important therapeutic agent. Additionally, it contains inulin, a polysaccharide that hydrolyzes into carbohydrates, along with a high content of essential vitamins such as B-complex, K, and E, and minerals like calcium, iron, and phosphorus. The plant also exhibits elevated concentrations of flavonoids [7].

Artichoke is recognized as one of the richest plant sources of non-enzymatic natural antioxidants, including vitamin C, polyphenols, tocopherols, phenolic acids, hydroxycinnamic acids, carotenoids, and flavonoids [8].

Mulberry (Morus alba L.) is a fast-growing deciduous plant that thrives in warm climates and is widely distributed across the globe. It belongs to the Moraceae family, which comprises two main genera: Morus (mulberries) and Ficus (figs). In Iraq, three species of mulberry are commonly found: white mulberry (Morus alba), which is highly prevalent, red mulberry (Morus nigra), and black mulberry (Morus rubra). The leaves of Morus alba serve as the primary food source for the silkworm (Bombyx mori L.) and are extensively used as fodder for ruminant animals, Mulberry leaves have garnered considerable medical interest due to their high content of bioactive compounds, particularly flavonoids and 1-deoxynojirimycin, a polyhydroxylated alkaloid known for its potent free radical scavenging activity [9], [10]. Furthermore, the leaves are rich in vitamins—especially vitamin A, B-complex, and vitamin D—as well as various organic acids and fatty acids [11].

The primary aim of this study was to investigate the individual and synergistic effects of artichoke and mulberry on the physiological performance of laying hens exposed to heat stress conditions.

Materials and Methods

This study was conducted to investigate the effect of supplementing artichoke leaf powder, mulberry leaf powder, and a mixture of both to the diets of laying hens subjected to heat stress at the onset of their production phase, focusing on their physiological performance.

A total of 144 laying hens, 23 weeks of age, were randomly allocated into six treatment groups. All experimental birds were fed a unified basal diet supplemented with artichoke powder, mulberry powder, and their mixture at concentrations of 0, 1, 1.5, 1, 1.5, and 1.5 + 1.5 g/kg feed for each respective treatment. The raw powder for each treatment was mixed with a weekly quantity of feed to ensure homogeneity of the added substance with the calculated feed amount for each treatment. The experimental birds were fed a laying hen diet containing 17.75% crude protein. The feed provided per hen ranged from 120 to 130 g/day, while water was supplied ad libitum. Table 1 details the composition of the experimental diet.

The lighting regimen during the study period exposed the birds to 16 hours of light and 8 hours of darkness per day. All birds were subjected to the same temperature conditions for equal durations (an average of 15 minutes every 6 hours). Accurate thermometers were strategically placed within the poultry house (three per house: beginning, middle, and end) to measure temperature. Temperatures were recorded in a dedicated log every 6 hours, resulting in four readings per day, and the average temperature was calculated weekly throughout the study.

Tabel 1. Feed Composition and Chemical Analysis.

Feed Ingredients:

Ingredients	Percentage(%)
Yellow Corn	36.0
Wheat	28.5
Soybean Meal (44% Crude Protein)	16.0
Protein Concentrate	10.0
Limestone	7.7
Sunflower Oil	1.5
Salt	0.3
Total	100

Chemical Analysis of the Diet:

Parameter	Value
Crude Protein (%)	17.75
Metabolizable Energy (kcal/kg feed)	2759
Energy-to-Protein Ratio	155
Lysine (%)	0.86
Methionine (%)	0.41
Methionine + Cysteine (%)	0.68
Calcium (%)	3.06
Phosphorus (%)	0.44

The chemical composition of the diet was determined according to the feedstuff analysis guidelines outlined in the National Research Council [12] report. The protein concentrate used in the formulation was produced by Provimi Company, originating from Jordan.

At the end of each study period, blood samples were collected via the brachial wing vein. Two birds were randomly selected from each replicate for each sampling event. The area surrounding the vein was first cleared of feathers, sterilized, and wiped with a cotton swab. The vein was then punctured using a 5 mL x 23 G plastic syringe.

The collected blood from each bird was portion was placed in tubes containing EDTA as an anticoagulant. These samples were then centrifuged at 3000 rpm to obtain blood plasma, which was subsequently stored in specialized glass tubes at -20°C for later biochemical analyses.

Biochemical parameters were determined as follows: Cholesterol concentration was measured using a specific kit supplied by Biolabo SA, following the method described by Richmond [13], Total protein concentration was determined using a specific kit supplied by Biolabo SA, France, according to the method outlined by Asatoor and King [14], Total glucose concentration was measured using a kit from the same supplier, following the method described by Wotton [15], Total calcium concentration in plasma was estimated using a kit from Biomerieux, according to the method described by Gindler and King [16], Total phosphorus concentration in plasma was determined using a kit supplied by Biolabo SA, France, following the method described by Tietz [17], The experiment was conducted using a Completely Randomized Design (CRD). The data were statistically analyzed using the Statistical Package for Social Sciences [18]. Significant differences among means were compared using Duncan's Multiple Range Test [19].

Results

The results presented in Table 2 to explain the impact of crude artichoke and mulberry powder, as well as their synergistic mixture, supplemented in the diets of heat-stressed laying hens on the concentrations of cholesterol, glucose, protein, calcium, and phosphorus across different study periods.

Cholesterol

The first period, cholesterol concentration Similar to highly significant superiority (P≤0.01) for treatments T4 and T5 compared to the other experimental groups. In the second period, treatments T3 and T1 demonstrated a significant advantage (P≤0.05) in cholesterol concentration when compared to the remaining treatments. For the third period, treatment T6 showed a highly significant superiority (P≤0.01) compared to the other experimental treatments. Finally, in the fourth period, treatments T5 and T4 exhibited a significant superiority (P≤0.05) compared to the other experimental groups.

Glucose

Regarding glucose concentration, treatments T1 and T4 displayed a highly significant superiority (P≤0.01) compared to other treatments for both the first and second periods. However, no significant was observed for any treatment in the third period. Their for, treatments T4, T6, and T5 sequentially showed a highly significant superiority (P≤0.01) when compared to the remaining study treatments.

Protein

The effect of treatment on protein concentration, treatments T2 and T5 demonstrated a highly significant superiority (P≤0.01) compared to the other experimental treatments during the first period. No significant treatment effect on protein concentration was observed for the second and fourth periods. For the third period, treatments T6, T4, and T5 sequentially showed a significant superiority (P≤0.05) compared to the remaining experimental treatments.

Calcium

The results also illustrate calcium concentration. In the first period, treatments T6, T5, and T4 sequentially exhibited a highly significant superiority (P≤0.01) when compared to the other treatments. During the second period, treatments T4, T6, T2, and T3 showed a highly significant superiority (P≤0.01) when compared to treatments T5 and T1. For the third period only, the supplemented treatments showed the same superiority at a significance level of (P≤0.01) when compared to the control treatment (T1). In the fourth period, treatments T6, T5, and T2 demonstrated the same superiority (P≤0.01) when compared to the remaining study treatments.

Phosphorus

Finally, the effect of treatment on phosphorus concentration is evident from the table. Supplemented treatments T5, T4, and T2 showed a highly significant superiority (P≤0.01) when comparing their results to the other experimental treatments. However, no significant superiority in phosphorus concentration was observed for the second period. For the third period, treatments T6 and T5 exhibited the same superiority (P≤0.01) over the other experimental treatments. Lastly, treatments T4 and T2 demonstrated a highly significant superiority (P≤0.01) when comparing their results to the remaining experimental treatments.

Table 2. Effect of adding crude artichoke and mulberry powder and their synergistic mixture on the levels of cholesterol, glucose, protein, calcium, and phosphorus in the blood plasma of laying hens exposed to heat stress (Mean ± Standard Error).

Traits / Period/Tret.		Chole.conce.	Glu.conce.	Prot.conce.	Calci.conce.	Phos. Conce.
Period1	T1	^{ab}180.14±0.13	^{a}193.12±1.12	^{c}3.55±0.32	^{ab}12.21±1.14	^{c}2.44±0.17
	T2	^{b}175.21±1.01	^{ab}185.32±1.41	^{a}4.21±0.68	^{b}11.95±1.41	^{a}2.93±1.03
	T3	^{b}176.22±1.02	^{ab}188.14±0.75	^{bc}3.61±0.22	^{b}11.84±1.32	^{b}2.83±1.12
	T4	^{a}188.44±0.88	^{a}191.14±0.63	^{b}3.66±0.65	^{a}12.44±1.41	^{a}3.05±1.01
	T5	^{a}183.22±0.33	^{ab}187.66±0.44	^{ab}3.78±0.63	^{a}13.05±1.02	^{a}2.98±1.18
	T6	^{b}178.25±1.08	^{b}181.22±1.14	^{b}3.75±0.45	^{a}13.25±1.02	^{b}2.85±1.23
Sig.		0.01	0.01	0.01	0.01	0.01
Period2	T1	^{a}188.12±1.12	^{a}198.32±2.01	3.12±0.10	^{b}13.44±2.03	3.55±1.66
	T2	^{c}175.36±2.06	183.14±2.31	3.14±0.65	^{a}14.25±1.33	3.87±1.11
	T3	^{a}190.22±1.01	^{b}185.65±0.98	3.22±1.04	^{a}14.18±1.66	3.65±1.74

	T4	[a]186.44±2.01	[a]189.12±0.65	3.18±0.33	[a]14.66±1.22	3.71±1.54
	T5	[bc]177.14±1.54	[c]181.24±1.02	3.29±1.44	[b]13.98±1.20	3.64±1.65
	T6	[b]178.66±1.65	[c]181.47±1.45	3.45±0.33	[a]14.25±2.03	3.66±1.63
Sig.		0.05	0.01	N.S	0.01	N.S
Period3	T1	[d]173.22±1.22	199.15±1.66	[b]4.65±1.02	[c]14.65±2.22	[c]4.61±2.04
	T2	[c]177.14±1.14	195.74±2.14	[b]4.73±2.11	[b]14.83±3.02	[c]4.68±2.11
	T3	[c]178.14±1.52	196.32±1.05	[ab]4.86±1.55	[b]14.98±2.03	[bc]4.75±2.33
	T4	[b]183.47±1.65	197.12±1.01	[a]5.01±2.01	[a]15.01±2.24	[a]5.11±3.04
	T5	[b]188.32±1.92	198.24±1.21	[ab]4.98±1.65	[a]15.24±2.36	[a]5.34±1.98
	T6	[a]198.14±1.65	196.14±1.33	[a]5.12±2.66	[b]14.90±1.65	[a]5.44±2.13
Sig.		0.01	N.S	0.05	0.01	0.01
Period4	T1	[ab]183.14±2.11	[b]225.24±4.14	5.42±2.22	[c]14.83±1.44	[c]3.28±1.54
	T2	[b]180.66±2.03	[c]218.66±5.04	5.89±2.77	[a]15.77±2.66	[a]4.08±2.04
	T3	[c]178.22±2.04	[bc]223.77±3.44	5.65±1.88	[b]14.98±2.44	[b]3.65±1.47
	T4	[a]185.17±1.99	[a]238.40±3.22	5.78±2.44	[b]15.14±2.33	[a]4.55±1.45
	T5	[a]189.55±2.33	[a]230.69±3.96	5.87±1.74	[a]15.88±1.65	[b]3.98±2.14
	T6	[b]180.17±2.28	[a]234.17±3.85	5.97±1.66	[a]16.33±2.55	[b]3.95±2.03
Sig.		0.05	0.01	N.S	0.01	0.01

Different letters within the same column indicate significant differences among the experimental treatments for each study period, Treatments T1, T2, T3, T4, and T5 received a control diet supplemented with crude mulberry leaf powder, artichoke leaf powder, and a synergistic mixture thereof at the following ratios (0, 1 g/kg feed of mulberry leaf powder, 1.5 g/kg feed of mulberry leaf powder, 1 g/kg feed of artichoke leaf powder, 1.5 g/kg feed of artichoke leaf powder, 1.5 g mulberry leaf powder + 1.5 g artichoke leaf powder/kg feed), respectively, (**) Represents highly significant differences between experimental treatments at P≤0.01, (*) Represents significant differences between experimental treatments at P≤0.05, N.S. indicates no significant difference between experimental treatments, Abbreviations: Chole.conce: Cholesterol concentration Glu.conce: Glucose concentration Prot.conce: Protein concentration Calci.conce: Calcium concentration Phos. Conce: Phosphorus concentration.

Discussion

Stress in poultry typically arises when birds encounter environmental changes that disrupt their physiological homeostasis. This triggers the body to initiate mechanisms aimed at restoring internal balance. In the current study, dietary supplementation appears to play a significant role in mitigating the severity of heat stress. It is well-established that heat stress induces cellular oxidation, which in turn elevates blood plasma cholesterol levels. Therefore, the observed stabilization of cholesterol levels within normal ranges could be attributed to the presence of bioactive compounds in these plants, such as flavonoids and alkaloids. These compounds are known antioxidants that prevent the oxidation of lipid membranes. They achieve this by neutralizing the oxidation process due to their multiple hydroxyl groups, which donate hydrogen atoms to free radicals, thereby stabilizing them and halting cellular oxidation [20], [21], [22], [23], [24].

Regarding blood plasma glucose production, the observed stability of levels within the normal range, despite the birds' exposure to heat stress, indicates a state of resilience and resistance to stress in the birds. This stability can be explained by the consistent levels of ACTH hormone, which is responsible for glucose synthesis and its association with the adrenal cortex. Elevated levels of this hormone typically lead to increased protein catabolism and hepatic glycogenolysis [25], [26], [27], [28].

The maintenance of protein levels within the normal physiological range (3.5-6.5 mg/g) in blood plasma, despite the imposed heat stress, may be attributed to the rich protein content of these plants. This enhances their dietary protein contribution and subsequently elevates plasma protein levels. Furthermore, these plants are rich sources of vitamin C and carotenoids, which are crucial in protecting the body from oxidative damage caused by heat, The observed improvements in calcium and phosphorus levels in the blood plasma of heat-stressed birds can be attributed to the numerous

compounds within these plants that possess biological effects beneficial for overall bird health and improved digestion and absorption [26], [29], [30]. These include volatile oils, flavonoids, and tannins, which contribute to increased bioavailability of these elements, leading to higher concentrations in the blood plasma [31], [32].

Conclusion

This study concludes that the supplementation of crude artichoke and mulberry leaf powder, and their mixture, played a significant role in reducing the severity of heat stress imposed on the birds. Therefore, these dietary additives can be utilized in poultry diets to enhance growth performance and mitigate the negative impacts of heat stress.

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