Effect of adding beetroot powder (*Beta vulgaris* L.) to the diet on some blood biochemical parameters in older laying hens

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Abstract

This study aimed to evaluate the effect of adding beetroot powder (Beta vulgaris L.) to the diet on certain blood biochemical parameters in older laying hens. One hundred and forty 51-week-old Lohmann Brown Classic laying hens were randomly assigned to five dietary treatments: a control, and treatments supplemented with beetroot powder at 0.5%, 1.0%, 1.5%, and 2.0%, with four replicates per treatment, each containing seven hens. The experiment lasted 140 days. At 70 weeks of age, blood samples were drawn from the brachial vein of two birds from each replicate and placed in anticoagulant-free tubes to obtain serum. Analysis was performed at the Central Laboratory of the University of Baghdad. The results showed a significant improvement ($P \leq 0.05$) in glucose, total protein, and globulin concentrations in the beetroot powder supplementation treatments, with treatments T3 and T5 registering the highest values compared to the control treatment. The control treatment also showed a significant increase ($P \leq 0.05$) in liver enzyme activity (ALP and ALT) compared to the supplementation treatments. Furthermore, statistical analysis revealed a significant advantage ($P \leq 0.05$) of the 2.0% beetroot powder treatment in estrogen and progesterone concentrations compared with the control treatment. The study concludes that adding beetroot powder to the diets of older laying hens, particularly at a concentration of 2.0%, improves Specific blood biochemical parameters and enhances liver function and sex hormone levels.

Keywords: Beetroot powder, laying hens, blood parameters, liver enzymes, sex hormones.

Introduction

Laying hens are among the most rapidly growing livestock species, as they provide consumers with eggs, that offer high-value protein at an affordable price across all segments of society [1]. With the increasing global population, the demand for such protein sources has grown. Commercial laying hens are characterized by egg production throughout their life cycle, with production cycles now extending to more than 100 weeks. The economic benefits of continuous production for a longer period include a reduction in cost and environmental impact per egg. However, there are challenges related to egg quality as laying hens age [2]. Due to physiological changes, egg production and quality decline with age. The reproductive organs, including the ovary and oviduct, are the main targets of aging. Ovarian aging is characterized by granulocyte death, chronic inflammation, oxidative stress, and abnormal hormonal responses. All these factors lead to a decrease in egg production [4,3]. Therefore, during the last productive period of egg laying, the percentage of broken eggs increases due to shell thickness resulting from a decrease in the amount of calcium available due to impaired intestinal absorption. The production of deformed eggs also increases compared to peak production [6,5]. Therefore, maintaining continuous egg laying in older hens while maintaining high quality is crucial, as hens that produce around 500 eggs over 90-100 weeks lead to a

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reduction in costs and a decrease in waste of natural resources [7]. Feed additives to the diet of hens laying eggs in the later stages of production can help maintain egg production and quality. Feed additives are an effective and practical way to improve egg-laying performance in the late egg-laying period, increasing economic benefits and enhancing the productivity of laying hens. Using natural feed additives rich in antioxidants as alternatives to antibiotics and synthetic supplements helps animals resist excess free radicals and supports their physiological functions [8, 9, 10]. Several studies have shown that adding certain natural supplements (PFAs) to chicken feed has a positive effect on egg production, weight, quality, and overall health. One such supplement is beetroot (Beta vulgaris L.), which belongs to the Chenopodiaceae family. Red beetroot has received increasing attention in recent years as a functional food due to its physiological and biological effects [11]. Its anti-inflammatory properties and content of betalains, betaxanthins, and other pigments have been demonstrated. The phytochemicals it contains, such as carotenoids, polyphenols, and flavonoids, have numerous health benefits [12]. Furthermore, beetroot is a potent antioxidant, which enhances the body's ability to produce blood, strengthens the immune system, and increases red blood cell count. Therefore, it has been used to prevent lipid peroxidation, boost immunity, improve bowel motility, and facilitate digestion in both humans and animals [13, 14]. The high fiber content in beetroot also has positive effects on intestinal function, preventing constipation and helping to lower cholesterol levels [15].

[1] Note: When different levels of beetroot powder were added to the feed of 120 Isa Brown laying hens aged 30 weeks, an addition of up to 1% increased hemoglobin and hematocrit levels and maintained them within normal ranges. In addition, additions of 0.5% and 1.5% red and white blood cell levels under normal conditions.

Given the reasons mentioned above, this study aimed to evaluate the effect of beetroot powder at graded levels in the feel on the performance and vital functions of older laying hens raised in a floor rearing system.

Material and Methods

1. Experimental Design

This study aimed to evaluate the effect of adding red beetroot powder (*Beta vulgaris rubra*) to the feed on the blood biochemical parameters of 51-week-old laying hens. The experiment was conducted on a private laying hen farm in Al-Ma'amra, Hilla, Babylon Governorate, Iraq, The experiment lasted 20 weeks (December 14, 2024 – May 2, 2025), following a two-week acclimatization period (December 1–13, 2024). preceded the experiment. One hundred and forty 51-week-old Lohmann Brown Classic (brown-shell) laying hens were randomly assigned to five feeding groups, with four replicates per group and seven hens per replicate. The experimental groups were arranged as follows:

• Group 1 (T1): Control feed (no beetroot powder).

• Group 2 (T2): Base feed + 5 g of beetroot powder/kg feed (0.5%). • Group T3: Base feed + 10 g of beetroot powder/kg feed (1.0%).

• Group T4: Base feed + 15 g of beetroot powder/kg feed (1.5%).

ISSN 2072-3857

• Group T5: Base feed + 20 g of beetroot powder/kg feed (2.0%).

At the end of the fifth period, at 70 weeks of age, blood samples were taken from two birds randomly from each replicate, and blood was drawn from the wing vein. Blood samples were placed in a tube without anticoagulant to obtain serum. The blood components were separated using a centrifuge at 3000 rpm for 15 minutes. After plasma separation, the samples were frozen (-20°C) for subsequent laboratory analysis. This analysis included glucose concentration, total protein, albumin, globulin, uric acid, enzymes (AST, ALT, ALP), estrogen, and progesterone. Biochemical analyses were performed at the University of Baghdad.

Feed Composition

All feeds were formulated to be equal in calories and nitrogen, in accordance with the National Research Councils recommendations [16] for laying hens. The basic feed composition is shown in Table 1.

Table 1: Feed ingredients and nutritional composition (percentage of dry matter).

%	Feed ingredient
55.0	Yellow corn
5.5	Wheat bran
24.0	Soybean meal (46% CP)
2.5	Protein premix 2.5%
1.3	Vegetable oil
10.85	Limestone
0.5	Dicalcium phosphate (21% P)
0.1	DL-Methionine
0.05	L-Lysine
0.1	Salt
0.1	Mycotoxin binder
100	Total
Calculated nutrient composition:	
2723	Metabolizable energy (kcal/kg feed)
16.00	Crude protein (%)
3.95	Calcium (%)
0.37	Available phosphorus (%)

The calculated chemical composition of the basal diet, derived from the formulated feed ingredients, was metabolizable energy 2723 kcal/kg, crude protein 16%, calcium 3.95%, and available phosphorus 0.37%. The protein premix (ProVimi, Netherlands) contained per kg: 5.9% crude protein and 3600 kcal ME. It also included 6.4% Ca, 5.7% P, and 6.5% Na. Additionally, it had 4000 mg Fe, 2800 mg Zn, and 600 mg Cu. Other components were 8.35 mg Co, 60 mg I, and 10 mg Se. The premix provided 5.9% methionine, 1.5% lysine, and 1200 mg niacin. It also contained 400,000 IU vitamin A, 140,000 IU vitamin D_3 , and 2000 mg vitamin E. Furthermore, it had 100 mg vitamin K, 90 mg vitamin B_1 , 160 mg vitamin B_2 , 200 mg vitamin B_6 , and 1000 µg vitamin $B_{1\ 2}$.

Preparation and Chemical Analysis of Beetroot Powder

Fresh red beetroot (Beta vulgaris L.) roots were washed, chopped, dried in the shade, and then ground into a fine powder. The chemical composition of the beetroot powder was determined at the Animal Resources Laboratory, Ministry of Agriculture - Quality Control Department, Baghdad, using the procedures described in Manual [17], as shown in Table 2.

Table (2). Chemical composition of beetroot powder (percentage of dry matter)

%	Component
5.37	Moisture
10.50	Crude protein
0.20	Ether extract (Fat)
11.80	Crude fiber
8.50	Ash

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63.63	Nitrogen-free extract (Carbohydrates)
2232	Metabolizable energy (kcal/kg)

Experiment Management and Data Collection

All hens were kept in identical environmental and sanitary conditions. The lighting program consisted of 16 hours of light and 8 hours of darkness per day, with unlimited access to feed and water.

2. Beetroot Powder Preparation

Fresh beetroot was purchased from local markets and thoroughly washed with tap water. It was then peeled and grated into small pieces. The grated beetroot was placed on trays and air-dried at 30°C until completely dry. The dried beetroot was then ground into a fine powder using an electric grinder and stored in paper bags at 20-22°C until used in the experimental rations.

3. Feed Formulation and Feed Management

The experimental base ration was prepared at Al-Ghadir Feed Mill (Babylon, Iraq). The hens received standard laying hen feed throughout the experiment. The feed was hand-fed at specific times (115 g/hen/day). Beetroot powder was mixed daily with the feed in particular proportions for each treatment.

4. Management and Housing Conditions

The chickens were housed on a litter-covered floor, in accordance with standard management practices. Lighting was on for 16 hours and off for 8 hours daily. Feed and water were continuously available, and all hygiene and safety procedures were strictly followed throughout the study period.

5. Statistical Analysis

All data were analyzed using the General Linear Model (GLM) procedure in SAS software [18], and the treatment means were compared using Duncans multiple range test [19] at a statistical significance level ($P \leq 0.05$).

Results and Discussion

Effect of Adding Beetroot Powder on Glucose, Uric Acid, Protein, Albumin, and Globulin Concentrations

Table 3 shows the effect of adding beetroot powder to laying hen diets on glucose, uric acid, protein, albumin, and globulin concentrations. A statistically significant interaction ($P \leq 0.05$) was observed among the experimental treatments in glucose, total protein, and globulin concentrations. Treatment T3 recorded the highest glucose concentration, while the two treatments that received the highest level of beetroot powder also recorded the highest total protein and globulin concentrations. [11] No significant differences were observed between the groups in serum glucose levels when beetroot powder was

administered to laying quail. The high content of neopetanin in beetroot, a betalain breakdown product, reduced the post-meal glucose response and insulin secretion, indicating a decreased need for insulin [20]. Furthermore, significant improvements in protein levels were

observed in the treatments. Beetroot contributes to high egg production in hens.

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Table (3) shows the effect of adding beetroot powder on glucose (mg/dl), uric acid (mg/dl), protein (g /L), albumin (g /L), and globulin (g/L) concentrations.

Uric Acid (mg/dl)	Albumin/ Globulin Ratio	Globulin (g/L)	Albumin (g/L)	Protein (g/L)	Glucose (mg/dl)	Treatments
0.36± 5.60	0.03± 0.573	29.82 1.18±ab	17.05 0.14±	46.80 1.03±ab	207.22 5.53±b	T1
2.33± 6.67	0.09± 0.562	28.00 0.75±ab	15.60 2.07±	43.55 1.35± b	214.95 6.43±ab	T2
0.80± 5.13	0.02± 0.681	25.20 0.98±b	17.15 0.43±	42.40 1.38± b	238.66 11.92±a	T3
0.45± 5.43	0.03± 0.567	33.95 2.45±a	19.10 0.23±	53.05 2.68±a	227.14 9.02±ab	T4
0.22± 5.26	0.02± 0.629	32.50 0.40±a	20.45 ±0.60	52.95 1.01±a	232.87 3.75±ab	T5
N.S.	N.S.	*	N.S.	*	*	Significance

Different superscript letters within the same column indicate significant differences ($p \leq 0.05$). * = Significant; N.S = Not significant.

Effect of Adding Beetroot Powder on AST (IU/L), ALT (IU/L), and ALP (IU/L) Enzyme Concentrations

Table 4 shows the effect of adding beetroot powder to laying hen diets on the concentration of AST, ALT, and ALP enzyme activity (international units/L) in serum (mean ± standard error). The beetroot powder treatments showed a decrease in ALT (alanine transaminase) activity compared to the control treatment. T5 hens treated with the highest level of beetroot powder exhibited the lowest ALP concentration among the experimental treatments. No significant differences were observed between the experimental treatments in AST (aspartate transaminase) activity. These results are consistent with [9], who followed a significant superiority ($P \leq 0.05$) of the control group in ALT and AST concentrations compared to the beetroot treatments in the broiler group. Betalains are protective antioxidants. Effective, as it acts as a metal chelator or can interrupt chain oxidative radical reactions and activate antioxidant defense mechanisms in vivo [21]. Betalain's ability to remove free radicals and its high antioxidant activity are related to the presence of hydroxyphenolic groups in its structure [22].

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Table 4 Effect of adding beetroot powder on the concentrations of the enzyme AST (IU/L), ALT (IU/L), and ALP (IU/L)

(IU/L) AST	(IU/L) ALT	(IU/L) ALP	Treatments
3.21± 174.00	a 6.67± 11.67	ab 77.02± 204.00	T1
12.70± 200.67	b 0.33± 5.33	a 82.48± 419.00	T2
16.16± 192.67	ab 1.15± 7.00	ab 110.85± 384.33	T3
26.88± 216.00	ab 0.58± 7.00	ab 58.10± 335.00	T4
17.38± 211.00	ab 0.88± 6.33	b 23.86± 190.00	T5
N.S.	*	*	Significance

Different superscript letters within the same column indicate significant differences ($p \leq 0.05$). * = Significant; N.S = Not significant.

Effect of Adding Beetroot Powder on Estrogen and Progesterone Concentrations (ng/ml)

Table 5 shows the effect of adding beetroot powder to laying hen diets on estrogen and progesterone concentrations. Statistical analysis revealed a significant ($P \leq 0.05$) increase in estrogen concentration in groups T2 and T5 compared with the other groups. Regarding progesterone, treatments T2, T4, and T5 significantly outperformed the control group ($P \leq 0.05$), which recorded the lowest progesterone concentration. Beetroot contains essential bioactive compounds such as betalains [23], phenolic compounds [24], carotenoids [25], nitrates, saponins, polyphenols, flavonoids [26], and ascorbic acid [27]. Adding beetroot to Adding beetroot to the feed may improve reproductive performance in older hens, partly by reducing stress. Oxidative stress on the reproductive organs [3]

Table 5: Effect of Adding Beetroot Powder on Estrogen and Progesterone Concentrations (ng/ml)

Progesterone (ng/ml)	Estrogen (ng/ml)	Treatments
0.01± 0.713 b	1.65± 0.373 b	T1
0.08± 0.880 a	13.45± 0.448 a	T2
0.03± 0.810 ab	8.15± 0.373 b	T3
0.04± 0.830 a	1.15± 0.366 b	T4
0.05± 0.865 a	15.80± 0.438 a	T5
*	*	Significance

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Different superscript letters within the same column indicate significant differences ($p \leq 0.05$). * = Significant;

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