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The Effect of Adding Rosemary Powder on the Physiological and Biochemical Characteristics of Blood and Antioxidant Status of Female Quails

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A B S T R A C T

This study was conducted at the poultry farm of the Animal Production Department, College of Agriculture / University of Kirkuk, during the period from 01/02/2023 to 24/2/2023, to determine the effect of adding rosemary powder on the physiological and biochemical characteristics of blood and the status of antioxidants in the blood serum of female quails. 64 female Japanese quails, eight weeks old, were used. The birds were randomly distributed into four treatments, each treatment had four replicates, and each replicate had four female Japanese quails. The birds were fed free feed, and the treatments were distributed as follows: The first treatment was a control treatment, i.e., without addition, the second treatment added 0.25% rosemary powder, the third treatment added 0.50% rosemary powder, and the fourth treatment added 0.75% rosemary powder. The results of the statistical analysis led to: The results led to an improvement in the percentage of antioxidants in the blood serum, as well as a significant decrease in the percentage of blood fats (cholesterol HDL-LDL-VLDL and also in the percentage of liver enzymes compared to the control treatment.

Introduction

Poultry meat and eggs are famous protein foods all over the world because they are cheap, high in nutrients, easy to digest, and taste great (Ameen et al., 2023). Poultry production, especially in developing countries, is one of the fastest-growing agricultural sectors in the world. The poultry sector is growing all the time because of the rising population and demand for poultry production. When breeders make a high-quality diet, they need to be very careful because infectious diseases can easily affect poultry production. Medicinal plants or herbs are good alternatives that can be used to help poultry grow (Nawzad et al., 2024).

The broiler industry has witnessed a significant the growth rate is high and the feed conversion efficiency is high as a result development of breeding techniques and genetic improvement, and this leads to the emergence of unintended negative consequences such as increased appetite and excessive feed intake due to the nature of free-range feeding of broilers (Tallentire. et al., 2016) (Hamady et. al., 2019). Recently, herb extracts have been fed as nontraditional feed additives to replace the growth promoters and antibiotics. The use of antibiotics has been restricted in many parts of the world, so scientists are turning to the use of medicinal plants (Ammar et al., 2020). Medicinal plants are used as raw materials in the manufacture of medication and now play a large role in the production of industrial products (Janghan et al., 2023). It is also used to produce some of the main chemical compounds used in the pharmaceutical business. These substances include flavonoids, glycosides, polyphenols, and saponins, all of which have therapeutic qualities (Nguyen et al., 2020; Merzah & Ali, 2021).

The World Health Organisation estimates that 80% of therapeutic plants have medical benefits; most of these benefits come from the extracts' or components' actions as growth promoters, antibiotics, and antibacterial agents. Antioxidants and antifungal agents (Ali & Al-Shuhaib, 2021). Furthermore, because of their immunostimulant, antibacterial, and antioxidant properties, plant essential oils have also been shown to inhibit infections. As a spice and flavouring agent in food preparation (Al-Kaissy et al., 2023). Medicinal plants such as crocin are considered to be a strong and direct antioxidant and a protective factor against damage resulting from internal oxidation of nucleic acids.

This study aims to use the antioxidant crocin in the pharmaceutical treatment of various types of diseases. Many studies have proven that it is used as a treatment for human diseases, and it is also very useful as a food additive for animals and poultry as a strong antioxidant to protect cells from oxidative damage (Mhamad & Palani, 2025). Rosemary (*Rosmarinus officinalis* L.) is an aromatic and medicinal plant that is frequently used in traditional prescriptions. It belongs to the evergreen mint family and is native to the Mediterranean region. Therefore, this study aimed to investigate the effect of rosemary powder on the physiological characteristics, antioxidant status, and blood biochemistry of female quails raised at high altitudes. Rosemary is a small shrub with branched, narrow, and sticky leaves. The phytochemicals in rosemary are mainly phenolic compounds, di- and triterpenes, including rosmarinic acid, camphor, caffeic acid, ursolic acid, carnosic acid, and carnosol (Yao et al., 2023).

Since some soils lack some elements, reducing the impact of mineral inputs on agricultural soil is an important strategy to protect agricultural lands and ensure food safety. This is also suitable for use as a fertilizer for plants and has no harmful effects on the environment. Its levels are within the limits permitted by the Institute of Medicine (United States), taking into account that the introduction of heavy metals into the soil and the food chain can have a potential negative impact on environmental quality and on animal and human health. This comes through food additives for animals, such as medicinal plants as antioxidants, and others (Palani et al., 2022). Rosemary and its constituents were known to have powerful antioxidant activity, antimicrobial, antiviral, antiinflammatory, and anticarcinogenic activities. Also, it can delay the rancidity in poultry products. Since quail is a small bird with great resistance to harsh environmental conditions and diseases, the experiment was conducted on Japanese quail (ELnaggar et al., 2016).

METHODS

This study was conducted in one of the poultry farms affiliated with the College of Agriculture, University of Kirkuk. This research aims to know the effect of adding rosemary powder on the physiological and biochemical characteristics of blood and the status of antioxidants in the blood serum of female quails. 64 female Japanese quails at the age of eight weeks were used. The birds were randomly distributed into four treatments, each treatment had four replicates, and each replicate had four female Japanese quails. The birds were transferred to five-storey batteries, each floor containing three cages designated for raising these birds. The dimensions of each cage are 40 x 30 x 20 cm, made of plastic mesh. Feed and water were provided freely to the birds. The calculation of the reading of nutritional transaction relationships was based on the clear structure according to what was stated in the National Research Council (N.R.C.1994) and as shown in Table (1). for two weeks as a preparatory period of 16 weeks for the female quails to get used to the new feed60-watt lamps were used to light the hall according to the lighting system of 17 hours of light and 7 hours of darkness during 24 hours.

The treatments were as follows: The first treatment (control) included the experimental feed without any additives. The second feed included the experimental feed with the addition of 0.25% of rosemary powder. The third feed included the experimental feed with the addition of 0.50% of rosemary powder. The fourth feed included the experimental feed with the addition of 0.75% of rosemary powder.

Preparation of Rosemary Powder

A quantity of rosemary was taken and dried properly, then ground using an electric grinder, and then added to the experimental feed.

Studied Traits

Blood samples were collected from Japanese quail at 16 weeks of experiment by taking samples from three replicates of each treatment, Two birds from each replicate, (Six birds from each treatment) randomly from the wing vein, to perform blood tests which included cellular blood parameters calculated according to method described by (Archer, 2016). The biochemical blood tests included the measurement of Glucose, total Protein, Albumin, Cholesterol, Triglycerides (TG), HDL, LDL, VLDL, Enzymes indicative of liver function, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline Phosphatase (ALP). Total antioxidant capacity was determined according to(Koracevic et al., 2011), Superoxide dismutase activity (Misra et al., 2011), Glutathione peroxidase activity (Paglia et al., 2011), and Glutathione activity (Ellman et al, 2015). Malondialdehyde (Hermandez et al, 2014; Guidet and Shah, 2018).

Statistical Analysis

Following blood sample collection, the data were combined and subjected to statistical analysis. A one-way analysis of variance (ANOVA) was employed within a Completely Randomized Design (CRD) framework. Data analysis was performed using the SAS statistical software (version 9.1) [SAS, 2012]. The significant differences between the treatment means were tested using Duncan's multiple range test (Duncan, 1955) at 0.05 and 0.01 levels of significance.

Table 1. Production feed used in the experiment

Feed material	%
the corn yellow	36.6
the Wheat	12
The barley	12.82
Soybean (44% protein)	25.93
Protein center	2.4
Limestone	9.25
The Vegetable oil	1.0
The Total	100
The chemical analysis	
The Representative energy (kilocalorie/kg feed)	2700
Crude protein (%)	17
Crude fiber (%)	3.67
Calcium (%)	4.14
(%) Available phosphorus	0.42
methionine + cysteine %	0.71
DCAB) Dietary Cation-Anion Balance (mg/kg)	202.43
Choline(%)	0.17
Folic acid (mg/kg)	0.54
Klycine(%)	0.73
Glycine+serine(%)	1.58
Histidine(%)	0.45
Isoleucine(%)	0.71
Leucine(%)	1.41
Lysine(%)	0.93
Methionine(%)	0.40
Cysteine(%)	0.29
Phenylalanine(%)	0.82
Tyrosine(%)	0.70
Phenylalanine + Tyrosine (%)	1.51
Threonine (%)	0.63
Tryptophan(%)	0.25
Valine(%)	0.81
Arginine(%)	1.06
Histidine(%)	0.45

•Protein concentrate from the Dutch company Profimi. Each kg contains: 5.9% crude protein, 3600 representative energy calories/kg, 6.4% calcium, 5.7% phosphorus, 6.5% sodium, 4000 mg/kg iron, 2800 mg/kg zinc, mg /kg 600 copper, 8.35 mg cobalt, 60 mg/kg iodine, 10 mg/kg selenium, 5.9% methionine, 1.5% lysine 5.9% methionine with cysteine, 1200 mg/kg niacin, 400,000 IU vitamin A, 140,000 IU vitamin D3, 2000 mg/kg E, 100 K, 90 mg/kg vitamin B1, 160 ppb vitamin B2, 200 mg/kg vitamin B6 and 1000 mg/kg vitamin B12**.**

RESULTS AND DISCUSSION

Table 2 shows that heat stress increases the production of reactive oxygen species (ROS), leading to tissue damage through lipid peroxidation. It was noted that rosemary contains antioxidant compounds such as phenols and terpenes, which enhance the body's ability to resist oxidative stress. Antioxidants are the first line of defense against oxidative stress caused by the accumulation of free radicals in the body[9]. By comparing the different treatments in this table:

1. GPX increased in T4 compared to T1 by approximately 69.2%.
2. GSH increased in T4 compared to T1 by about 4.45%.
3. SOD increased in T3 compared to T1 by approximately 79.4%.

4. MDA decreased in T4 compared to T1 by about 39%.
5. CAT decreased in T4 compared to T1 by 13.3%.

GPX (Glutathione Peroxidase): GPX is an essential enzyme in removing harmful free radicals like lipid peroxides. Its increase in T3 and T4 compared to T1 reflects the effect of rosemary in improving the body's detoxification ability, which may be attributed to the phenolic compounds in rosemary, such as carnosic acid and rosmarinic acid, known for their antioxidant properties. These compounds help boost antioxidant enzyme activity. The two previous studies indicated that increased GPX activity reduces the risk of diseases related to oxidative stress, such as inflammation and cancer.

GSH (Glutathione): Glutathione is a key factor in regulating oxidative stress within cells. The noticeable increase in T4 suggests that rosemary enhances the production or recycling of glutathione in the body, helping reduce oxidative damage. A decrease in GSH indicates acute oxidative stress, but in this study, its increase in T4 suggests that rosemary may enhance the production of this vital antioxidant, aligning with previous studies that showed plants rich in flavonoids and phenols contribute to boosting glutathione levels.

SOD (Superoxide Dismutase): T3 recorded the highest levels of SOD, indicating that rosemary may increase the activity of this enzyme, responsible for neutralizing harmful free radicals like superoxide anions. Increased SOD activity is associated with reduced oxidative damage in tissues, especially in the liver and muscles. The two previous studies noted that the intake of antioxidants from natural sources enhances SOD activity, improving the body's response to oxidative stress from high metabolic activity.

MDA (Malondialdehyde): MDA is a key indicator of lipid oxidation, and its high levels indicate cell damage due to oxidation. Its decrease in T4 and T3 reflects rosemary's protective effect against lipid oxidation, meaning that rosemary helps maintain cell membrane integrity and reduce tissue damage. The previous studies suggested that a decrease in MDA correlates with improved overall health, as lipid oxidation leads to many health issues, such as atherosclerosis and heart disease.

CAT (Catalase): The decrease in catalase levels in T4 may reflect the reduced need for this enzyme's activity in removing harmful hydrogen peroxide. Rosemary may improve the overall oxidative state by increasing the activity of other enzymes such as GPX and SOD.

Table 2. Effect of rosemary powder on the antioxidant status in the blood serum of female quails.

Tretment	GPX (mg/dl)	GSH	SOD	MDA (mmol/ml)	CAT (mmol/ml)
T1	0.26 c	929 d	197 b	9.59 a	7.56 a
T2	0.41 b	947 c	234 b	9.26 b	7.18 b
T3	0.43 a	957.5 b	353.5 a	7.66 c	4.39 d
T4	0.44 a	970.5 a	245.5 b	5.84 d	6.55 c

The different letters within one column indicate significant differences between the parameters ($p < 0.05$)

Table 3 shows that rosemary improves overall health by reducing harmful fats, consistent with the results showing a decrease in cholesterol and lipid levels. The percentage effects on lipids are significant as they reflect cardiovascular health:

1. Cholesterol decreased in T4 compared to T1 by 9.55%.
2. HDL decreased in T4 compared to T1 by 11.65%.
3. TG decreased in T4 compared to T1 by 14.5%.
4. VLDL decreased in T4 compared to T1 by 14.5%.
5. LDL decreased in T4 compared to T1 by 6.8%.

Cholesterol: The decrease in cholesterol in T4 compared to the control group indicates that rosemary may help regulate lipid levels in the body. This is attributed to the ability of phenolic compounds in rosemary to improve fat metabolism enzyme activity and reduce cholesterol production in the liver. Previous studies showed that rosemary reduces cholesterol absorption from the intestines due to its fiber and phytochemical content, aligning with the results here (Polat et al, 2011).

HDL (High-Density Lipoprotein): Although HDL is known as the “good cholesterol”, T4 saw a slight decrease in it. This may be due to an overall improvement in fat balance in the body. However, the general effect on HDL may require further research to determine whether these changes are harmful or beneficial in the long term.

TG (Triglycerides): The significant decrease in T4 reflects rosemary's effective role in reducing triglyceride levels, which are a major risk factor for heart and vascular diseases. It is believed that rosemary reduces fat accumulation in the body by increasing fat metabolism enzyme activity.

VLDL (Very Low-Density Lipoprotein): The continuous decrease in VLDL with increasing doses of rosemary reflects its protective effect against harmful fat accumulation. This protein is typically associated with triglyceride transport in the blood, so its decrease is an indicator of overall improved lipid health.

LDL (Low-Density Lipoprotein): LDL, the “bad cholesterol”, is responsible for fat accumulation in arteries. Although the changes in its levels were minor, the general trend of its decrease in T4 supports the idea that rosemary helps improve heart health by reducing the risk of fat buildup in the arteries.

Table 3. Effect of rosemary powder on the lipid status in the blood serum of female Quails

Tretment	cholestrol	HDL	TG	vldl	ldl(mg/dl)
T1	277.50	99.6	175.5	35.1	142.7
	a	a	a	a	a
T2	267.5	89 .0	157.5	31.5	147.0
	b	b	b	b	a
T3	565.5	91.6	151.5	30.3	143.6
	b	b	c	c	a
T4	251	88	150	30.0	133.0
	c	b	c	c	b

The different letters within one column indicate significant differences between the parameters ($p < 0.05$)

Table 4 shows that heat stress is a primary factor in liver damage and an increase in liver enzyme levels, such as ALT and AST, which are linked to liver damage. The study pointed out that rosemary reduces the damage caused by oxidative stress and protects the liver from injury. Liver enzymes are key indicators of liver health and function:

1. ALT decreased in T4 compared to T1 by 14.7%.
2. AST decreased in T4 compared to T1 by 20.3%.
3. ALP increased in T3 compared to T1 by 20.5%.

ALT and AST (Transaminases): The significant decrease in ALT and AST in T4 compared to the control group suggests that rosemary has a protective effect on liver cells, as elevated levels of these enzymes usually indicate liver damage. Rosemary contains compounds that help reduce inflammation and tissue damage, which is consistent with previous studies on the effect of antioxidant-rich plants on liver health.

ALP (Alkaline Phosphatase): The slight increase in ALP in T3 compared to the decrease in T4 may reflect different dosage effects on liver function. While an increase in ALP may indicate heightened liver or bone activity, its decrease in T4 could indicate stability in liver function. Since AST and ALT are generally believed to be liver enzymes that exacerbate liver damage (hepatocellular degeneration), their decreased serum concentrations may indicate that rosemary has a liver-protective effect (Hermandez et al, 2014).

Table 4. Effect of rosemary powder on liver enzymes in the blood serum of female quails

Tretment	ALT	AST	ALP (U/L)
T1	49.7	70.8	54.9
	a	a	b
T2	48.1	59.3	42.1
	b	b	d
T3	46.1	57.9	66.2
	c	c	a
T4	42.4	56.5	47.8
	d	d	c

The different letters within one column indicate significant differences between the parameters ($p < 0.05$)

Table 5 indicates that rosemary oil helps improve immune response and increases protein concentrations in the blood. Biochemical traits are indicators of overall health:

1. Glucose showed almost no difference between T4 and T1.
2. Protein increased in T2 compared to T1 by 13.8%.
3. Albumin decreased in T3 compared to T1 by 3.01%.
4. Globulin increased in T3 compared to T1 by 1.24%.

Glucose: The lack of significant differences between treatments suggests that rosemary does not have a major effect on glucose metabolism in quails. This may require further research into its role in regulating blood sugar levels.

Total Protein: The notable increase in T2 may indicate that rosemary enhances protein production in the body, improving overall health and supporting growth. Compounds in rosemary may improve the body's ability to utilize nutrients and increase protein synthesis. This illustrates how fowl can store proteins in their tissues after they have consumed more than their allotted amount (Ghazalah & Ali, 2018).

Albumin and Globulin: The changes in albumin and globulin levels may reflect rosemary's effects on immune response and nutritional status, as albumin is indicative of the body's nutritional state, while globulin is linked to immune response.

Table 5. Effect of rosemary powder on the biochemical characteristics of the blood serum of female quails

Tretment	Protein (mg/dl)	protein (mg/dl)	Albumen (mg/dl)	Globulin (mg/dl)
T1	365.50	4.13	1.66	2.42
	a	b	a	b
T2	343.00	4.70	1.58	2.41
	b	a	b	b
T3	368.75	4.06	1.61	2.45
	a	b	c	a
T4	366.90	4.04	1.64	2.37
	a	b	b	c

The different letters within one column indicate significant differences between the parameters ($p < 0.05$)

Table 6 shows that rosemary powder can reduce reproductive hormone levels like LH and FSH, negatively affecting fertility. However, rosemary was found to improve fertility by reducing oxidative damage. Reproductive hormones play a critical role in regulating fertility:

1. LH increased in T2 compared to T1 by 12%.
2. FSH decreased in T4 compared to T1 by 70.2%.
3. Estradiol increased in T2 compared to T1 by 73.8%.

Table 6. Effect of rosemary powder on reproductive hormones in the blood serum of female quails

Tretment	LH	FSH	Estradiol (u/l)
T1	0.25 ab	1.04 a	200.5 b
T2	0.28 a	0.71 ab	348.5 a
T3	0.21 b	0.59 ab	201.5 b
T4	0.26 a	0.31 b	149.0 b

The different letters within one column indicate significant differences between the parameters ($p < 0.05$)

LH and FSH (Luteinizing Hormone and Follicle-Stimulating Hormone): The increase in LH and FSH in T2 suggests that rosemary may enhance reproductive activity and improve ovulation chances. This aligns with some studies that showed rosemary contains plant compounds that stimulate hormonal activity. Estradiol: The increase in T2 indicates that rosemary may boost sexual activity, and this effect could be related to rosemary's compounds, which stimulate estradiol secretion, enhancing fertility and increasing the chances of reproduction.

Conclusion

Adding rosemary powder at rates of 0.25%, 0.50% and 0.75% improved the status of antioxidants and lipids in blood serum, as well as improving the level of liver enzymes and reproductive hormones, thus improving the physiological characteristics of female quails.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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