



Special Issue:

Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

The Modification of Broiler Physiological and Immunological Traits Caused by the Administration of Varying Concentrations of Oregano Leaf Aqueous Extract (*Origanum vulgare*)

HASAN SA JAWAD^{1*}, NIHAD ABDUL-LATEEF ALI², GALIB A. AL-KASSIE³, ABDUL-RAZZAK L. AL-RUBAIE³, LOKMAN IH⁴

¹Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq; ²Department of Animal Production, College of Agriculture, Al-Qasim Green University, Babylon, Iraq; ³National University of Science and Technology, Dhi-Qar, Iraq; ⁴Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, UPM, Malaysia.

Abstract | This experiment was conducted at poultry field in the Agriculture College- Al Qasim Green University during 18 September 2020 until 23 October 2020. It was used 240 of broiler chicks Ross 308, chicks were randomly distributed to four experimental treatments, where added the aqueous extract of Oregano leaves at levels (0, 3, 5, 7 ml/L). All of the histological traits were studied such as the villus height, the crypts depth, the percentage of the villus height to the crypts depth and immunological traits are both the immunity directed against Newcastle disease and the test of the relative hypersensitivity in the wattles and relative weight and the Fabricia gland index, and biochemical traits, such as concentration of glucose, cholesterol, triglyceride, globulin, albumin, and total protein.

Keywords | Aqueous extract, *Origanum vulgare*, Small intestine tissue, Immunological, Broiler chicks

Received | July 08, 2024; **Accepted** | September 17, 2024; **Published** | November 20, 2024

***Correspondence** | Hasan SA Jawad, Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq; Email: dr.hassan198366@yahoo.com, hasan.s@coagri.uobaghdad.edu.iq

Citation | Jawad HSA, Ali NA-L, Al-Kassie GA, Al-Rubaie A-RL, Lokman IH (2024). The modification of broiler physiological and immunological traits caused by the administration of varying concentrations of oregano leaf aqueous extract (*Origanum vulgare*). J. Anim. Health Prod. 12(s1): 113-119.

DOI | <http://dx.doi.org/10.17582/journal.jahp/2024/12.s1.113.119>

ISSN (Online) | 2308-2801



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INTRODUCTION

Since this knowledge is vital to the prosperity of the chicken business, there has been a surge in interest in the investigation of natural phytochemical compounds used as substitutes for synthetic ingredients in food production (Abbas et al. (2018); Hasan et al., 2020; Choi et al., 2002; Hamzah and Nihad, 2022). The oregano plant, known by its scientific name *Origanum Vulgare*, is a perennial herb plant that may be found growing naturally in the Mediterranean Sea sink as well as in America and Europe. Its scientific name is *Origanum Vulgare*. Because it is a natural source of antibacterial compounds, it is regarded to be one of the most significant plants in the field of

medicine. Phenols and flavonoids are the most significant of its numerous useful components (Burt and Reinders, 2003; Fotea et al., 2010), but it also has many other useful components. In addition to its possible medicinal and aromatic uses, the leaves of this plant contain around 5-25% volatile oils, which have long been employed in cooking to prevent food from oxidizing too quickly. This is because its leaves have a high concentration of volatile oils. The most significant and effective components of it are called developing peaks (Ariana et al., 2002; Najafi and Torki, 2010; Nihad et al., 2021). Other useful components include compounds derived from spruce gum, which inhibit the growth of harmful germs such as positive and negative bacteria (Menati et al., 2018; Surai and Kukenko,

All of the components of oregano are having an effect and operating collectively as a unit. The production of body salts is the primary focus of its activity. In addition to their role in improving the function of the stomach, these components may also have a diuretic effect. It has been discovered that the presence of these components results in an increase in the number of hydrolyzed enzymes that are secreted as well as an improvement in the function of the liver, both of which are necessary for proper digestion. In addition to this, research has shown that oregano can improve the function of the immune system (Tako et al., 2004; Al-Kaissy et al., 2023; Abbas and Jawad, 2023). On the contrary to other antibiotics such as tetracycline, and other drugs that have not been tested yet, the trial study on oregano indicated that bacteria actors on a wide range of pathogens. Abbas et al. (2018) apart from mentioned macro-nutrients one may say that active components (such as phenolic and vitamin E) may also add to an improvement of the functioning of the body. Hence, after this process, such avian developed massive amounts of antibodies, which their immune system was successfully able to withstand the destructive pathogens (Saad et al., 2000; Samar et al., 2020; Ali and Jawad, 2021; Nihad et al., 2023), therefore they imply that the subsequent intake of medicinal plants and oregano in chicken diet may lead to the formation of more number of Lymphocyte cells that are less suppressed hence activating the killer cells that produce antibodies. Lastly, this was materialized as Tannin again joined forces by augmenting the production of white blood cells and their release to the blood of the chicken. This is the term that is used in medicinal science to describe the production of red blood cells. Based on the study summarized above, the consumption of oregano leaves within greater doses has been asserted to have a significant effect by elevating the level of glucose that is found in the bloodstream. Or, alternatively, the word owiege could be branding the leaves of oregano, that, like the ones of glucocorticoids, stimulate the blood sugar plasma to increase. This is in contrast with gluconeogenesis, a process in which sugar can be formed from sugar sources other than carbohydrates, sugar sources instead of carbohydrates.

Metabolism rises leading to an aggrandizement in albumen concentration in the blood plasma which is a constituent of protein in the blood due to the consumption of it. Not only this but it also transports other hormones including thyroxine, glucose, amino acids, vitamins, and certain metals. A rise in the degree of oregano results in a fall in the number of cholesterol depositions in the blood serum. The fact that oregano stimulates metabolic activity that often results in higher production levels of the thyroid hormone thyroxine, it becomes effective in the dropping of triglycerides and cholesterol levels in the blood plasma, also becsaludise the

role of Vitamin A which makes its relative consolidation in this process. Hence, this study focuses on finding out the physiological impact and textural effect that a water extract from the fresh oregano leaves has on chicken.

MATERIALS AND METHODS

This experiment was conducted at poultry field in the Agriculture College-Al-Qasim Green University during 18 September 2020 until 23 October 2020. It was used 240 of broiler chicks Ross 308, with one day age and 40 g weight. The chicks were breaded in a hall with 40 m × 10 m dimension in four-floor cages. Each floor contains a cage of 1.5×1 m, the chicks were randomly distributed in three experimental treatments with 60 chicks per treatment and three replicates of treatment (20 chick/replicate) as following:

First treatment: (treatment of control without any addition). The second treatment: add of the aqueous extract of Oregano leaves with a concentration of (3 ml extract/liter) drinking water. The third treatment: add aqueous extract of Oregano leaves with a concentration of (5 ml extract/liter) drinking water. The fourth treatment: Add the aqueous extract of Oregano leaves with a concentration of (7 ml extract/liter) drinking water. The chicks were fed until the age of 22 days on the initiator's ration and from 23 days until the age of marketing 35 days on the growth ration as shown in Table 1, feed was provided in free form.

PREPARATION OF THE EXTRACTION

The dried Oregano leaves were brought from local markets in Babhdad province and tested using a small electric mill to be as powder, and then prepare the extract according to the method of (Nbras and Nihad, 2023) modified by mixing the amount of dry powder with the amount of distilled water by 1 g: 2 ml using the electric mixer and display the solution to 60 °C for one hour and leave the solution for 24 hours at room temperature, The solution was then filtered by several layers of Sterilizer medical gauze to be ready for use in the experiment.

STUDIED TRAITS

HISTOLOGICAL TRAITS

At the end of the experiment, at the age of 35 days, 3 birds of each replicate were slaughtered by 9 birds per treatment. Three parts of the small intestine were taken (Duodenum, Jejunum, Ileum). The length of the cut was 4 cm. After the contents were removed and washed several times with water, the samples were prepared according to (Tako et al., 2004) method. The histological slides were examined using a compound microscope and all measurements were recorded using the Ocular Micrometer with a 400x magnification force after calibration to the stage micrometer and the villus length, crypts depth and the

ratio of villus length to crypts depth (v/c) was estimated.

Table 1: Composition of basal diet.

Feed material	Starter diet (1-21) % days	Finished diet (22-35) days %
Yellow corn	48.2	58.7
Local wheat	8	7.5
Soybean meal (44% protein)	28.5	20.5
Protein concentrate*	10	10
Vegetable oil (sunflower)	4	2.5
limestone	1	0.5
Table salt	0.3	0.3
total summation	100 %	100%
** Calculated chemical analysis		
Representative energy (kcal/kg)	3079.85	3102.6
Crude protein (%)	21.56	18.87
Lysine (%)	1.04	0.85
Methionine+Cycine (%)	0.455	0.42
Crude fiber %	3.54	3.2
Calcium (%)	1.28	1.07
Ready phosphorus (%)	0.42	0.41

* A protein concentrate of Belgian origin, one kilogram of which contains: 2200 kilocalories of representative energy, 40% crude protein, 8% fat, 3.5% fiber, 25% ash, 8% calcium, 3.1 ready-made phosphorus, 1.2% lysine, 1.2% Methionine, 1.8% Methionine + 70 mg B, 30 mg Vitamin E, 1 300 mg Vitamin D, 2500 IU A, 3 Cysteine, 2% Chlorine, 10,000 IU 12 mg Folic acid, B 250 plus B, 120 mg Pantothenic acid, 400 mg niacin, 50 mg Vitamin B 2, 5000 mg Choline Closed, 450 mg Iron, 70 mg Copper, 600 mg Biotin, 1000 mg Vitamin E, 750 mg Manganese, 5 mg Iodine, 1 gm Cobalt And antioxidants. ** According to the chemical composition according to the analyzes of feed materials mentioned in the NRC (1994).

The villus length was measured from the top of the villus until it is associated with the crypts, the crypts depth is the distance of immersing the neighboring villus (Tako et al., 2004).

IMMUNE TRAITS

The immunological traits were both the Enzyme-linked immunosorbent assay testing (ELISA) method, the test of hypersensitivity in the wattles (Surai and Kuklenko, 2000). The relative weight of Fabricia’s gland and Fabricia’s index, as indicated by the researchers (Ulaiwi, 2023). Serum was stored in other sterilized tubes at a temperature of -18 °C for the purpose of conducting laboratory analyzes and in accordance with instructions attached to kits for the purpose of estimating the concentration of glucose, cholesterol, triglycerides, globolins, albumins and total protein (Monika and Warhorn, 2012).

STATISTICAL ANALYSIS

The completely randomized design (CRD) was used to study the effect of different coefficients in the studied traits. Significant differences between the averages were measured by a Duncan test (1955) multidimensional test under a significant level of 0.05 SAS (2012) was used in statistical analysis.

RESULTS AND DISCUSSION

HISTOLOGICAL TRAITS

Tables 2, 3, 4 show the effect of using different concentrations of Oregano leaves extract in both the height of the villus, the depth of the crypts and the percentage of the height of the villus to the depth of the crypts in the parts of the mall intestine (Duodenum, Jejunum, Ileum) for broiler chicks. It is noted that the treatment T4 was significantly increased (P≤0.05) in the villus height in both the Duodenum and Jejunum compared to the treatment T3 improvement in turn significantly (P≤0.05) compared to treatment T2, which also improvement significantly (P≤0.05). While treatment T4 (P≤0.05) was improvement in the villus height in ileum and depth of crypts in duodenum than in T2, which was significantly improvement (P≤0.05) compared to control treatment. The treatment of T4 was significantly improvement (P≤0.05) compared to the control treatment at the depth of the crypts and the percentage of the height of the villus to the depth of the crypts in both the Jejunum and ileum. There were no significant differences between all the parameters in the ratio of the height of the villus to the depth of the crypts in the Duodenum.

Table 2: Effect of the use of different concentrations of the aqueous extract of Oregano leaves in the villus height, the crypts depth and the percentage of the villus height to the crypts depth in the Duodenum of the broiler chicks ± the standard error.

Parameters	Duodenum		
	Villus height micrometer	Crypt depth micrometer	Ratio of Villus height to crypt depth
T1	93.13±1.13 d	10.35±0.02 c	8.99±0.02
T2	108.85±2.36 c	11.16±0.47 b	9.30±0.02
T3	106.33±1.89 b	12.41±0.50 ab	8.56±0.09
T4	109.73±1.46 a	14.82±0.50 a	7.40±011
Significant level	*	*	N.S

T1 First treatment: control treatment. T2 Second treatment: 3 ml aqueous extract of the Oregano/liter drinking water. T3 Third treatment: 5 ml aqueous extract of the Oregano/liter drinking water. T4 Fourth treatment: 7 ml aqueous extract of the Oregano/liter drinking water. N.S indicates that there are no significant differences between the averages of treatment. * The different letters within the same column indicate significant differences between totals at the probability level of 0.05.

Table 3: Effect of the use of different concentrations of the aqueous extract of oregano leaves in the villus height, the crypts depth and the percentage of the villus height to the crypts depth in the Jejunum of the broiler chicks±the standard error.

Parameters	Jejunum		
	Villus height micrometer	Crypt depth micrometer	Ratio of Villus height to crypt depth
T1	78.26±0.63d	12.74±0.11 b	6.14±0.009
T2	83.41±0.35c	13.22±0.04ab	6.30±0.011
T3	86.84±0.33b	13.41±0.03ab	6.44±0.007
T4	90.37±0.51a	14.61±0.03a	6.18±0.006
Significant * level		*	N.S

T1 First treatment: control treatment. T2 Second treatment: 3 ml aqueous extract of the Oregano/liter drinking water. T3 Third treatment: 5 ml aqueous extract of the Oregano / liter drinking water. T4 Fourth treatment: 7 ml aqueous extract of the Oregano/liter drinking water. N.S indicates that there are no significant differences between the averages of treatment. * The different letters within the same column indicate significant differences between totals at the probability level of 0.05.

Table 4: Effect of the use of different concentrations of the aqueous extract of oregano leaves in the villus height, the crypts depth and the percentage of the villus height to the crypts depth in the ileum of the broiler chicks±the standard error.

Parameters	Ileum		
	Villus height micrometer	Crypt depth micrometer	Ratio of Villus height to crypt depth
T1	31.43±0.27 c	7.51±0.56 b	4.18±0.006 b
T2	36.71±0.16 b	7.72±0.27 ab	4.75±0.005 ab
T3	37.45±0.12 ab	7.81±0.15 ab	4.79±0.005 ab
T4	42.00±0.15 a	8.16±0.18 a	5.14±0.004 a
Significant level	*	*	*

T1 First treatment: control treatment. T2 Second treatment: 3 ml aqueous extract of the Oregano/liter drinking water. T3 Third treatment: 5 ml aqueous extract of the Oregano/liter drinking water. T4 Fourth treatment: 7 ml aqueous extract of the Oregano/liter drinking water. * The different letters within the same column indicate significant differences between totals at the probability level of 0.05.

Table 5: Effect of the use of different concentrations of the aqueous extract of Oregano leaves in the immune response to broiler chicks ± the standard error.

Parameters	Celluar immunity DTH	Newcastle immunity ELISA	Relative weight of fabricia gland	Fabricia guide
T1	0.154± 0.001 c	2543.3±89.37 c	0.069±0.001 c	1.000± 0.014 d
T2	0.189±0.002 b	2821.4±56.66 b	0.096±0.001 b	1.39±0.010 c
T3	0.229±0.001 ab	2935±40.12 ab	0.110±0.001 ab	1.59±0.012 b
T4	0.241±0.001 a	2945.6±77.06 a	0.116±0.001 a	1.68±0.011 a
Significant level	*	*	*	*

T1 First treatment: control treatment. T2 Second treatment: 3 ml aqueous extract of the Oregano/liter drinking water. T3 Third treatment: 5 ml aqueous extract of the Oregano/liter drinking water. T4 Fourth treatment: 7 ml aqueous extract of the Oregano/ liter drinking water. * The different letters within the same column indicate significant differences between totals at the probability level of 0.05.

IMMUNE TRAITS

Table 5 illustrates the effect of using different concentrations of the aqueous extract for Oregano leaves in the immune response to broiler chicks. It is noted that the treatment T4 was significantly excelled ($P \leq 0.05$) compared to the treatment T2 excelled in turn significantly ($P \leq 0.05$) compared to control treatment in both cellular immunity (DTH) and immunity directed against Newcastle disease and the relative weight of Fabricia's gland, while no significant differences were observed between T3 and T4, As for the Fabricia gland index, the treatment T4 was significantly excelled ($P \leq 0.05$) compared to the treatment T3 which excelled in turn significantly ($P \leq 0.05$) compared to treatment T2, which was significantly excelled compared to the control treatment.

Table 6 shows the effect of using different concentrations of the aqueous extract for Oregano leaves in some of the biochemical traits for plasma of broiler chicks. There is significant decrease in both concentration, cholesterol and triglycerides in T4 treatment compared to T1 and T2 treatments with significant increase ($P \leq 0.05$) in the concentration of glucose, albumin, globulin and total protein in the same treatment compared to the two treatments T2 and T3 ($P \leq 0.05$) which are significantly excelled compared to control treatment.

HISTOLOGICAL TRAITS

The use of the aqueous extract of Oregano leaves, improved all the studied tissue traits in the intestines of broiler chicks. The containment of Oregano leaves on Oregano compounds, which has an important role in improving the health status of birds by increasing their resistance to pathogens and toxins, which led to increased cellular circulation, Which speeds up the regeneration process (Nbras and Nihad, 2023). The leaves of Oregano, like some medicinal plants contain some effective compounds such as aromatics and vegetable oils, which reduce the various intestinal infections as a result of its high activity to resist pathogenic bacteria, as well as

Table 6: Effect of the use of different concentrations of the aqueous extract of Oregano leaves in the concentration of glucose, cholesterol, triglyceride, albumin, globulin and total protein in plasma of broiler chicks ± the standard error.

Parameters	Glucose con- centration (mg/100ml)	Cholesterol concentration (mg/100ml)	Triglyceride concentration (mg/100ml)	Albumin concentration (mg/100ml)	globulin concentration (mg/100ml)	Total protein (mg/100ml)
T1	c 158.21±0.50	a 192.52±0.79	a 65.73±0.37	c 1.18±0.01	c 1.06±0.01	c 2.24±0.03
T2	bc164.73±0.56	b 179.76±0.41	b 59.44±0.13	b 1.37±0.01	b 1.28±0.01	b2.65±0.04
T3	ab172.55±1.11	b 174.83±0.16	bc56.79±0.40	b 1.46±0.02	b 1.35±0.02	b2.81±0.01
T4	a 184.69±0.93	c 166.17±0.33	c 53.90±0.05	a 1.61±0.01	a 1.51±0.01	a 3.12±0.01
Significant level	*	*	*	*	*	*

T1 First treatment: control treatment. T2 Second treatment: 2 ml aqueous extract of the Oregano/liter drinking water. T3 Third treatment: 4 ml aqueous extract of the Oregano/liter drinking water. T4 Fourth treatment: 6 ml aqueous extract of the Oregano/ liter drinking water. *the different letters within the same column indicate significant differences between totals at the probability level of 0.05.

the increase of mature enterocytes of which increases the villus height and the crypts depth and thus increase their absorption efficiency (Lokman et al., 2015).

IMMUNE TRAITS

Nihad et al. (2023) noted that Oregano has a significant inhibitory effect against pathogenic bacteria compared with antibiotics such as Tetracycline, Gentamycin and Ciprofloxacin due to its containment of some active compounds, essential oils, (Nbras and Nihad, 2023; Najeh et al., 2023) that Oregano has the ability to dissolve the internal membrane of pathogenic bacteria, which is carried out into the bacterial cell, Affecting their basic components. Morphological improvement in both cellular immunity (DTH), immunity against Newcastle disease and the relative weight of fabricia at the high levels of the aqueous extract of Oregano leaves may be due to the traits of the extracts which made it have a good and effective effect on the functions of the body. As well as contain compounds phenolic in addition to contain vitamin E, which makes the level of antibodies high and therefore the immunity of the body of birds are highly resistant to pathogens (Najafi and Torki, 2010; Layth and Nihad, 2023) noted that the use of medicinal plants, including Oregano, may improve the immune system of broiler chicks due to the development of the ratio between T lymphocytes cells assistance and inhibitory, Enhancing the activity of natural killer cells or activating B lymphocytes on the production of antibodies and stimulating the bone marrow to produce white blood cells and increase the production of some staph lymphocytes (Cytokines) of lymphocytes because of the effect of active compounds and most important resin materials such as tannin and thymine.

CONCLUSIONS AND RECOMMENDATIONS

The results showed a significant improvement ($P \leq 0.05$) for the treatment of the aqueous extract of high

concentration Oregano leaves (7 ml/L drinking water) in all studied traits compared to all study treatment.

ACKNOWLEDGMENTS

I give my great thanks to the Deanship of the college of Agriculture - Al Qasim Green University and the station of research and agricultural experiments to facilitate procedures for the scientific research of birds and feed. As well as the use of the poultry hall in this research.

NOVELTY STATEMENT

In order to understand the impact of aqueous extract from Oregano leaves (*Origanum vulgare*) in broiler chicken, a total of 240 broiler chicks (Ross 308)s were randomly distributed into four experimental treatments. Three groups were administered with an increasing concentrations of aqueous extract of Oregano leaves ((3, 5, and 7 ml/L) whereas a non-treated group was used a control. All of the histological traits (villus height, the crypts depth, and the percentage of the villus height to the crypts depth),immuno-logical traits (immunity directed against Newcastle disease, relative hypersensitivity in the wattles ,relative weight and the Fabricia gland index)and biochemical traits (con- centration of glucose, cholesterol, triglyceride, globulin, albumin, and total protein) were improved in treated com- pared to control group in a dose-dependent manner. These finding provided novel insights that water extract from the fresh oregano leaves can significantly improve the health and performance of broiler chickens

AUTHOR'S CONTRIBUTION

Hasan SA Jawad: conceived of the presented idea, inves- tigate and supervised the findings of this work, carried out the experiment, supervised the project.

Nihad Abdul-Lateef Ali: conceived of the presented idea,

verified the analytical methods, carried out the experiment, contributed to the final version of the manuscript.

Galib A. Al-Kassie: conceived of the presented idea, developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations.

Abdul-Razzak L. Al-Rubaie: conceived of the presented idea, developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations.

Lokman I.H.: conceived of the presented idea, developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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