



EFFECT OF ADDING DIFFERENT LEVELS OF *THYMUS VULGARIS* LEAVES POWDER TO THE DIET, ON BLOOD PHYSIOLOGICAL TRAITS AND MICROBIAL CONTENT OF THE GUT OF BROILER CHICKENS

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Abstract

This experiment was conducted in the field of Poultry birds belonging to Department of Animal Production, College of Agriculture, Al-Qasim Green University in order to study the Effect of adding different levels of *Thymus vulgaris* powder leaves to the diet on some traits blood physiological and microbial content of the gut for broiler chickens, with one-day age were used, where they were randomly divided into four treatments, with a rate of 45 birds per treatment and each treatment consisted of three replicates (15 birds per replicate). The treatments of experiment were as follows: First treatment (control) without adding *Thymus vulgaris* powder leaves to the diet, second treatment: Adding *Thymus vulgaris* powder leaves with amount of (2 g / kg feed), third treatment: Adding *Thymus vulgaris* powder leaves with amount of (4 g / kg feed) and treatment Fourth: Adding *Thymus vulgaris* powder leaves with amount of (6 g / kg feed). The experiment included the following traits: Concentration of glucose, total protein, the concentration of albumin, concentration of globulin, globulin/albumin (G / A) ratio, concentration of total cholesterol, concentration of triglyceride, concentration of high-density lipoprotein, concentration of low-density lipoprotein, concentration of very Low-density lipoprotein, estimating the total number of bacterial, estimating the total number of Coliform bacteria and estimating the total number of Lactobacillus bacteria. The results indicated that the adding of *Thymus vulgaris* leaves (4, 6 g / kg feed) led to increasing the concentration of high-density lipoproteins and concentration of globulins as well as a significant decrease in glucose, total cholesterol, triglycerides, and low-density lipoproteins and occurring a significant decrease in the logarithmic numbers for the total aerobic bacteria and Coliform bacteria with a significant increase in the logarithmic numbers for Lactobacillus bacteria in the Duodenum contents for the small intestine as well as in the Ceca compared to the control treatment. It is concluded from the current experiment that adding *Thymus vulgaris* powder leaves to the diet can lead to improving some physiological traits and microbial content for the digestive tract for broiler chickens.

Keywords: *Thymus vulgaris* leaves, physiological traits, microbial content ,broiler chickens.

Introduction

Modern science has proved by conclusive evidence that the plant kingdom is rich in by-products that characterized by its biological bioactivity and its physiological effect therapeutically against the incurable diseases that affect humans, animals, birds and other organisms (Abu Zeid, 2000). The cultivation of medicinal, aromatic plants and herbs has spread in most parts of the world and its uses have varied and its traits for its pharmacological effectiveness and the speed of healing for diseases without complications, whether used in the form of whole herbs or powders or pastes or capsules and others (Hussein, 1981). It has recently witnessed the introducing many medicinal plants in the feeding of broiler chickens and laying hens, including Parsley leaves (Nihad *et al.*, 2016), Moringa leaves (Aqeel *et al.*, 2018), white tea leaves (Nihad *et al.*, 2019) and laurel leaves (Nihad, 2020). One of these plants is *Thymus vulgaris*, Thyme plant is famous plant of the platoon oral abound grown generally in the Mediterranean basin countries and call it a recipe delightful mountains because perfumed mountains smart scent has a strong aromatic smell and taste spicy slightly bitter, and spread the cultivation of thyme in Jordan, Syria and northern Iraq is thyme *Thymus vulgaris* one of the important medicinal plants, one of the herbs that are characterized by being a natural source of antioxidants (Miura and Nakatani, 2000) contains basic materials antioxidant which phenols and flavonoids (Haraguchi *et al.*, 1996) and self-oxidation process Auto oxidation can stop or discourage the addition of anti materials-oxidant and is considered thyme one aromatic and medicinal plants which

are used in food to prevent self-oxidation (Yodium and Deans, 2000) and the effective portion and user medically in thyme is a securities and developing peaks floral where the leaves contain essential oils volatile oils by (5-25%) and contain this oil on about 55% of the phenolic substances delusional Thymol (44.58–58.1%) and Carvacrol (2.4– 4.2%) These compounds are highly anti-microbial who attributed them to the medical benefits of thyme (Bartosikova *et al.*, 2003), as well as the gummy resin materials resins and Tanin and Linoleic acid (Stahl-Biskup and Laakso, 1990) and thyme contain active substances Next : Thymol both types of phenols, a medically important and carvacrol, Borneol, menthone, pinene, cymene, linalool.

One of the main uses of the plant medical thyme leaves pointed (Manou *et al.*, 1998) to the thyme leaves ground used as a substance that has the properties of a portfolio of damage where used in keeping the pharmaceutical and cosmetic and add aromatic odor of medical preparation in addition to its role in protecting the skin to prevent bacteria growth as it works to inhibit bacterial positive and negative growth when the concentration thymol 38.60%, while fungi (Bruneton, 1999) were among the possibility of using the painkiller for colic intestinal to address the inflammation of the stomach and intestines and stomach ulcers thyme is the regulator of the functions of the digestive system is also used thymol internally repellent worms as well as used in the sterilization of the mouth, teeth and skin, which is an anti-inflammatory (Deans, 1987). In view of the above and the lack of research in the use of this plant in poultry diets, this study aims to determine the effect of *Thymus vulgaris* leaves plant added to

the diet on the physiological traits and microbial content of the gut for broiler chickens and to know the best percentages that can be used in the diet.

Materials and Methods

This study was conducted in the field of Poultry birds belonging to the Department of Animal Production, College of Agriculture, Al-Qasim Green University for the period from 15/4/2018 to 27/5/2018, which continued to 42 days. A 180 unsexed broiler chickens (Ross 380), with the average weight amounted to (39g/chick), were used. The chicks were reared in-ground cages, and the chicks were randomly distributed on 4 treatments, Each consists of 3 replicates where each replicates contains 15 chicks. Feeds were provided to the birds freely, where two diets were fed, initiator diet from (1 to 21 days) and final diet from 22 to 42 days. The *Thymus vulgaris* powder leaves were added to the diet (manual mixing) from the age of one day as follows: First treatment (control) without adding *Thymus vulgaris* powder leaves to the diet, second treatment: Adding *Thymus vulgaris* powder leaves with amount of (2 g / kg feed), third treatment: Adding *Thymus vulgaris* powder leaves with amount of (4 g / kg feed) and treatment Fourth: Adding *Thymus vulgaris* powder leaves with amount of (6 g/kg feed). The following traits were estimated at the end of the experiment which continued for five weeks: Concentration of glucose, total protein, the concentration of albumin, concentration of globulin, globulin/albumin (G / A) ratio, concentration of total cholesterol, concentration of triglyceride, concentration of high-density lipoprotein, concentration of low-density lipoprotein, concentration of

very Low-density lipoprotein, estimating the total number of bacterial, estimating the total number of Coliform bacteria and estimating the total number of Lactobacillus bacteria Blood samples were collected after slaughtering birds in tubes that did not contain anticoagulants. The blood plasma was separated by a centrifuge at a speed of (3000 rpm) for 15 min, and the serum was kept in clean tubes at 20 °C. A diagnostic kit (from Jordan origin) was used to measure cholesterol (mg / 100 ml) according to (Franey and Elias, 1968). as for the concentration of glucose, Kits from French origin was used and it was calculated according to the following equation:

Concentration of glucose (mg/dl)

$$= \frac{(A) \text{ sample}}{(A) \text{ Standard}} \times \text{Standard concentration (100 mg/dl)}$$

Triglycerides, low-density, high-density lipoproteins were estimated as mentioned in (AOAC, 1980), total protein (g / 100 ml) and uric acid (mg / 100 ml) based on the method in (Henry *et al.*, 1982). As for the concentration of globulin, it was calculated according to the following equation: (concentration of total protein - concentration of albumin). Table (1) shows the used feed materials and their calculated chemical composition during the experiment period. The Completely Randomized Design was used to study the effect of different treatments on the studied traits, Significant differences between the averages were compared using the Duncan's Multiple Range Test (Duncan, 1955) and the Statistical package for social sciences (SAS, 2010) was used to analyze the data.

Table 1: Percentage of Feed materials included in the formation of the Initial Diet and Final diet Used in the Experiment with the Calculated Chemical Composition for Both Diets.

Feed material	Initial diets (1-21 days)%	Final diets (22-42 days)%
yellow corn	48.2	58.7
Local wheat	8	7.5
Soybean meal (44% protein)	28.5	20.5
The concentrated Protein*	10	10
Vegetable oil (sunflower)	4	2.5
limestone	1	0.5
Food salt	0.3	0.3
Total	% 100	%100
The Calculated Chemical Analysis **		
Metabolic Energy (kg / kg)	3079.85	3102.6
crude protein (%)	21.56	18.87
Lysine (%)	1.04	0.85
Methionine + Cysteine (%)	0.455	0.42
Raw fiber%	3.54	3.2
Calcium (%)	1.28	1.07
phosphorus availability (%)	0.42	0.41

* the concentrated protein, each kilogram contains: 2200 kcal/kg metabolic energy, 40% crude protein, 8% fat, 3.5% fiber, 25% ash, 8% calcium, 3.1 phosphorus availability, 1.2% lysine, 1.2% Methionine, 1.8% Methionine + 70 mg, 30 mg vitamin B1, 300 mg vitamin E, 2500 IU D3, A cysteine, 2% chlorine, 10,000 IU 12 mg folic acid, 250 B12, B 120 mg pantothenic acid, 400 mg niacin, 50 mg vitamin B2 and 6, 5000 mg Choline chloride, 450 mg iron, 70 mg copper, 600 mg, C 600 mcg biotin, 1000 mg special vitamin, 750 manganese, 5 mg iodine, 1 g cobalt and antioxidants.

** The chemical composition was calculated according to the analysis of feed materials mentioned in (NRC, 1994).

Results and Discussions

Table (2) shows the effect of the adding *Thymus vulgaris* powder leaves to the diet on the biochemical traits of blood plasma for broiler chickens. Where the table shows significant differences between the treatments in the concentration of glucose in the blood of birds, where a

significant excelling ($P \leq 0.05$) in favor of the control treatment on the rest of the treatments, The results also indicated that there were no significant differences between the treatments in the concentration of total protein and albumin. As for the concentration of globulin, it is noticed from the results of the table that the treatments of *Thymus*

vulgaris leaves were significantly ($P \leq 0.05$) excelled on the control treatment (first). As for the G / A ratio, the control treatment recorded the highest G / A ratio, with a significant difference ($P \leq 0.05$) from the rest of the treatments. The decrease in the concentration of glucose in the treatments of *Thymus vulgaris* powder leaves may be due to an increase in proteins unassociated with Glutathione, which contributes to the metabolism of various nutrients in the blood of birds

including glucose (Thakare, 2004). The reasons for increasing the averages of globulin in the treatments of *Thymus vulgaris* powder leaves are due to the fact that they are considered medicinal plants and helping in improve digestion in birds. This contributes to an increase in nutrient content, including proteins associated with Glutathione in the liver, thus increasing the percentage of Y-Globulin protein in the blood (Gardzieta wska *et al.*, 2003).

Table 2 : Effect of adding *Thymus vulgaris* powder leaves to the diet on blood plasma traits for broiler chickens at the age of (6 weeks).

Treatments	Concentration of Glucose (mg / 100 ml)	Total protein (g / 100 ml)	Concentration of albumin (g / 100 ml)	Concentration of globulin (g / 100 ml)	G / A ratio
First treatment (control)	174.75± 4.85 a	5.65± 0.40	4.68± 0.37	0.97± 0.03 b	4.82± 0.005 a
Second treatment (2g <i>Thymus vulgaris</i> leaves)	154.25± 4.02 b	5.58± 0.09	4.48± 0.09	1.10± 0.01 a	4.07± 0.002 b
Third treatment (4 g <i>Thymus vulgaris</i> leaves)	155.95± 2.40 b	5.60± 0.33	4.45± 0.14	1.15± 0.08 a	3.87± 0.003 c
Fourth treatment (6 g <i>Thymus vulgaris</i> leaves)	155.70± 3.72 b	5.82± 0.09	4.47± 0.27	1.35± 0.02 a	3.31± 0.004 d
Significant level	*	N.S	N.S	*	*

NS: no significant difference between treatments * There were significant differences at the level of ($P \leq 0.05$)

Table (3) shows the effect of adding *Thymus vulgaris* powder leaves in the diet on the average concentration of cholesterol and blood fat for the broiler chickens. Where the results showed significant differences between the treatments in the concentration of cholesterol, triglycerides, and lipoproteins. It indicated a significant improvement in these traits except for the results of lipoproteins and very low density which did not show any significant differences between treatments and control treatment in the averages of this trait. The results showed that the treatments of *Thymus vulgaris* powder leaves (second, third and fourth) recorded a significant decrease in the concentration of total cholesterol, triglycerides, concentrations of low-density lipoprotein compared to the first treatment (control) which recorded the highest concentrations in these traits. As for the concentration of high-density lipoproteins, the fourth treatment (6 g *Thymus vulgaris* leaves) recorded significantly ($P \leq 0.05$) the highest concentration compared to the control treatment, which recorded the lowest concentration for this trait, while there were no significant differences between the second and third treatments from control treatment on the

one hand and the fourth treatment on the other. The decrease in concentrations of cholesterol, triglycerides, and low-density lipoprotein for the treatments of *Thymus vulgaris* powder leaves may be due to the role of *Thymus vulgaris* powder leaves, which reduce enzymes that are excreted from the liver and form fatty acids. Or, the decrease in the concentration of triglyceride may be due to inhibiting the Acetyl-CoA Synthetase, which is an essential enzyme in the synthesis of fatty acids (Haraguchi *et al.*, 1996). As for the significant increase in the concentration of high-density lipoproteins for the fourth treatment of *Thymus vulgaris* leaves may be attributed to the role of Lysine and Polyunsaturated fatty acids found in *Thymus vulgaris* leaves, which accelerates the association of high-density lipoproteins with liver membranes and rid the body from harmful low-density lipoprotein molecules (Ali *et al.*, 2007). In addition to the role of active substances in *Thymus vulgaris* leaves, which have the ability to inhibit the activity of free radicals because they have an effective role of antioxidant within the body as considering it one of the most important types of natural antioxidants (Arora *et al.*, 2000).

Table 3: Effect of adding *Thymus vulgaris* powder leaves to the diet on the average concentration of cholesterol and blood lipid for broiler chickens at the age of (6 weeks).

Treatments	Concentration of Cholesterol (mg / 100 ml)	Concentration of Triglyceride (mg / 100 ml)	Concentration of high-density lipoproteins (mg / 100 ml)	Concentration of low-density lipoproteins (mg / 100 ml)	Concentration of very low-density lipoproteins (mg / 100 ml)
First treatment (control)	7.01±189.7 a	0.8 ± 94.90 a	1.66±81.9 b	7.01±102.63a	2.56±18.98
Second treatment (2g <i>Thymus vulgaris</i> leaves)	8.32±170.3 b	84.00 2.14± b	±88.7 1.24 ab	8.32 ± 64.8 b	1.57±16.8
Third treatment (4 g <i>Thymus vulgaris</i> leaves)	7.53±159.31b	76.30± 2.54 b	±86.1 1.10 ab	7.53±57.95 b	1.88±15.26
Fourth treatment (6 g <i>Thymus vulgaris</i> leaves)	8.64±154.06b	74.63± 5.51 b	±93.7 1.94 a	8.64±45.43 b	2.23±14.93
Significant level	*	*	*	*	N.S

NS: no significant difference between treatments * There were significant differences at the level of ($P \leq 0.05$)

Table (4) shows the effect of adding *Thymus vulgaris* powder leaves in the diet on the logarithmic numbers of total aerobic bacteria, Coliform bacteria and Lactobacillus bacteria (cfu/gr) of the duodenal and ceca contents for broiler chickens, where the table shows a significant decrease in the total numbers for the aerobic bacteria and Coliform bacteria in the duodenum and cecum in favor of the fourth treatment compared to the other treatments. It was showed a significant ($P \leq 0.05$) decrease in the total number for aerobic bacteria and Coliform bacteria for the two treatments (second and third) compared to the control treatment (first). It also shows a significant superiority ($P \leq 0.05$) in favor of the fourth treatment in the number of Lactobacillus bacteria for the two regions of the duodenum and cecum compared to other treatments. Significant superiority ($P \leq 0.05$) continued in the logarithmic number of Lactobacillus in favor of the treatments (third and second) compared to the first treatment (control). While there were

no significant differences between the two treatments (second and third) in the two regions of the duodenum and cecum in the numbers of the aerobic bacteria and Coliform bacteria and Lactobacillus bacteria. The treatments of *Thymus vulgaris* leaves added to the poultry diets gave the best results in the decrease of the total numbers of the aerobic bacteria and Coliform bacteria with an increase in the numbers of Lactobacillus. This may be due to the fact that *Thymus vulgaris* leaves contain phenolic compounds that have antibacterial activity against the positive and negative pathogenic bacteria for Cram (Al-Shahat, 2000). Most of the active compounds affect the structural and functional traits for the cell membrane in microorganisms and affect the permeability of the membranes, thus affect the performance and vitality of those organisms, so Lactobacillus bacteria has excelled in their number on the pathogenic bacteria, Thus it supports microbiological balance within the intestines (Erturk *et al.*, 2006).

Table 4: Effect of adding *Thymus vulgaris* powder leaves to the diet on the logarithmic numbers for the total aerobic bacteria, coliform bacteria, and Lactobacillus bacteria for the contents of the duodenum and the ceca for broiler chickens (average \pm Standard error).

Treatments	Duodenum			Ceca		
	Total aerobic bacteria	Coliform bacteria	Lactobacillus bacteria	Total aerobic bacteria	Coliform bacteria	Lactobacillus bacteria
First treatment (control)	0.06 \pm 5.27 a	0.13 \pm 11.31 a	0.05 \pm 3.73 c	0.04 \pm 3.97 a	0.08 \pm 7.46 a	0.03 \pm 2.91 c
Second treatment (2g <i>Thymus vulgaris</i> leaves)	0.05 \pm 4.61 b	0.11 \pm 10.82 b	0.04 \pm 4.14 b	0.03 \pm 3.41 b	0.07 \pm 7.06 b	0.02 \pm 3.47 b
Third treatment (4 g <i>Thymus vulgaris</i> leaves)	0.04 \pm 4.57 b	0.09 \pm 10.73 b	0.03 \pm 4.22 b	0.03 \pm 3.35 b	0.07 \pm 6.98 b	0.03 \pm 3.56 b
Fourth treatment (6 g <i>Thymus vulgaris</i> leaves)	0.05 \pm 4.35 c	0.09 \pm 10.58 c	0.04 \pm 4.53 a	0.04 \pm 3.16 c	0.05 \pm 6.71 c	0.02 \pm 3.66 a
Significant level	*	*	*	*	*	*

• There were significant differences at the level of ($P \leq 0.05$)

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