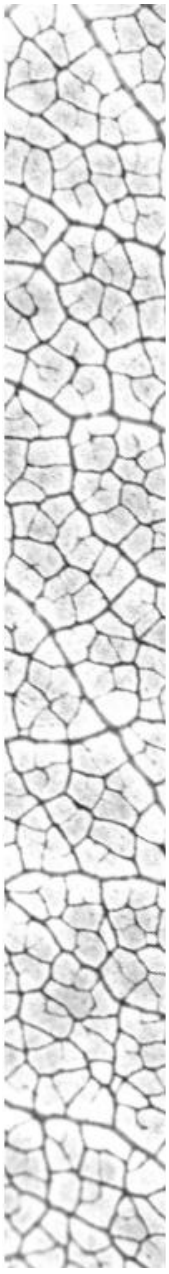

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Response of immune system and microbial content of gut to feeding with different levels of crushed laurel leaves (*Laurus nobilis*) in broiler chickens

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Abstract
This experiment aimed to study the effect of dietary inclusion of different levels of crushed laurel leaves (*Laurus nobilis*) on the immune responses and microbial content of gut in broiler chickens. A total of 180 unsexed one-day-old broiler chickens were randomly divided into four treatments (45 birds per treatment) and each treatment consisted of three replicates (15 birds per replicate). The treatment groups were: control; without adding crushed laurel leaves to the diet, second treatment; Adding 1 g/kg feed crushed laurel leaves to diet, third treatment: Adding crushed laurel leaves with amount of (2 g / kg feed) and treatment Fourth: Adding crushed laurel leaves with amount of (3 g / kg feed). The experiment included the following traits: antibody titer against Newcastle disease, investigating sensitivity against Newcastle disease, the relative weight of bursa of Fabricius, bursa of Fabricius index, 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 adding the crushed laurel leaves significantly improved cellular immunity and antibody titer against Newcastle disease as well as relative weight of bursa of Fabricius and bursa of Fabricius index. 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 that the addition of crushed laurel leaves to the diet can improve the immune response and microbial content of digestive tract in broiler chickens.

Keywords: broiler chicken, immune response, laurel leaves, microbial content

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INTRODUCTION

The poultry industry is considered with great importance in the agricultural sector and in raising the level of living for humans because of its production from white meat, eggs, and other by-products. Poultry has excellent food conversion efficiency compared to other farm animals. We have recently witnessed the introduction of 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) and white tea leaves (Nihad et al. 2019). One of these plants is *Laurus nobilis*, the English name for the bay tree, which is also called sweet bay tree or Grecian tree. The laurel tree has olive-like fruits that distinguish it with its dark brown color and be with clusters form. It is classified as an evergreen herbal medicinal plant belonging to the Lauraceae family, where it is used in treating gastrointestinal diseases and stomach ulcers (Kivcak and Mert 2002). i *Laurus nobilis* is extracted from the fruits of laurel volatile oil (Eugenol), Its percentage about (1 to 3%) in addition to possessing many active substances such as Monoterpenes, cinnamaldehyde, thymol, and carvacrol. As well as, it has the antioxidant activity because it contains the phenolic compounds (Erturk et al. 2006). The laurel leaves are used as fresh or dried aromatic plants (as a spice) in cooking to take advantage of its distinctive aroma and flavor (Nazia and Perween 2006). Karaalp and Genc (2013) found that the use of laurel leaves in quail diet for egg production led to lower the concentration of malondialdehyde in serum compared to the control treatment. Musa et al. (2011) reported that addition of laurel leaves to the quail diet improved egg production and egg weight and lower the concentrations of cholesterol and triglyceride compared

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Table 1. Feed ingredients and chemical composition of starter and finisher diet

Feed ingredients	starter diet (1-21 days)	finisher diet (22-35 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
Crude fiber (%)	3.54	3.2
Calcium (%)	1.28	1.07
Available phosphorus (%)	0.42	0.41

* 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, 30 mg vitamin B1, 300 mg vitamin E, 2500 IU D3, , 2% chlorine, 10,000 IU folic acid, 250 B12, 120 mg pantothenic acid, 400 mg niacin, 50 mg vitamin B2 and B6, 5000 mg Choline chloride, 450 mg iron, 70 mg copper, 600 mg 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).

to the control treatment. In view of the above and the lack of research in the use of this plant in poultry diets, this study aimed to determine the effect of laurel leaves added to the diet on the immune response and microbial content of gut in the broiler chickens (Ross 308) and to know the best percentages that can be used in the diet.

MATERIALS AND METHODS

Bioassay Technique

This study was conducted in the field of Poultry birds belonging to the Department of Animal Production at the College of Agriculture, Al-Qasim Green University for the period from 21/3/2019 to 25/4/2019, which continued to 35 days. A total of 180 unsexed broiler chickens (Ross 308 strain), with an average weight of 40 g, were reared in-ground cages. The chicks were randomly distributed to four treatments group; each consists of three replicates (15 chicks/replicate). The birds were fed *ad libitum* with starter diet from 1 to 21 days and finisher diet from 22 to 35 days of age as shown in **Table 1**. Dried samples of Laurel leaves were prepared and kept them in sealed bags the Laurel leaves powder samples were ground with an electric mill and the ratios assessed in the research were mixed with diets, in preparation for submission to broiler in this method there is no vaccine or antibiotic was used rearing period then the crushed laurel leaves were added to the diet (manual mixing) from the age of one day as follows: First treatment (T1control) the diet without crushed laurel leaves, second treatment (T2) : crushed laurel leaves mixed with amount of (1 g/kg feed), third treatment(T3) : crushed laurel leaves mixed with amount of (2 g/kg feed) and treatment the fourth treatment (T4): crushed laurel leaves mixed with amount of (3 g/kg feed). The following parameters were measured at age of 35 days:

Antibody Titer against Newcastle Disease (ELISA)

Specific antibodies were estimated quantitatively by ELISA according to (Voller et al. 1977). After incubating the test serum in an antigen-coated polystyrene tube or plate, enzyme labelled anti-immunoglobulin is added and the enzyme then remaining in the tube or plate after washing provides a measure of the amount of specific antibody in the serum. The test relies on the insolubilization of antigens by passive adsorption to a solid phase.

Bacteria Estimation

Estimating the total number of bacteria, estimating the total number of coliform bacteria and estimating the total number of *Lactobacillus* bacteria were carried out in the laboratory of the College of Science / University of Babylon. Small piece of intestine contents (duodenum and cecum) was taken for each bird and added to 9 ml of pre-prepared peptone solution so that the initial dilution was 10^{-1} and the solution was stored in the refrigerator at a temperature of 4 ° C for further microbial examination (Mukhtar et al 2018).

Estimation of Coliform Bacteria

Four concentrations from peptone solution were used in this exterminate (10-1,10-2, 10-3 and 10-4 to estimate the total number of total coliform bacteria Pour plate count method mentioned in (APHA 1978,) was used the plates were kept inverted at a temperature of 37 o for 48 hours from the bowel sample (colony / gm), APHA (American Public Health Association 1978).

Estimate the *Lactobacillus* Bacteria

Six glass tubes were prepared containing 9 ml of peptone solution and took 1 ml of the first dilution solution 10^{-1} to the first tube to be dilution 10^{-2} and took 1 ml of it to the second tube and so on to the sixth tube to be the dilution ratio 10^{-7} and the Pour plate count method mentioned in (speak 1984, using a solid MRS culture medium was used to estimate the total number of L. acidophilus by transferring 1 ml to each decimal diluent by sterile pipette to two layers of Duplex sterile Petri dishes. Each 15 ml dish of sterile culture medium MRS Agar pre-prepared and preserved in Water bath at 46 ° C then mix the bacterial trap with the medium to cultivate well by moving the dish quietly in all directions. After the hardening of the culture medium, the dishes were kept inverted at a temperature of 37 ° C. For 48 hours, the growth of the colonies were investigated, and the cultivar dish with the optimal decimal dilution were selected in preparing the colonies for it and then hit the inverted inverting to get the number of colonies Germs / gm from the bowel sample (colony / gm).

Table 2. Effect of adding crushed laurel leaves to the diet on the immune response of broiler chickens

Treatments	Cellular Immunity (DTH) mean±SE	Newcastle immunity (ELISA) mean±SE	The relative weight of bursa of Fabricius mean±SE	Bursa of Fabricius index mean±SE
T1 (control)	0.015 ± 0.168 c	230.4 ± 2719.6 c	0.001 ± 0.065 c	0 ± 1.000 d
T2 (1 g laurel leaves)	0.013 ± 0.214 b	215.5 ± 2846.7 b	0.001 ± 0.098 b	0.013 ± 1.508 c
T3 (2 g laurel leaves)	0.011 ± 0.217 b	203.8 ± 2855.7 b	0.002 ± 0.101 b	0.011 ± 1.554 b
T4 (3 g laurel leaves)	0.011 ± 0.243 a	200.9 ± 2917.2 a	0.001 ± 0.124 a	0.011 ± 1.908 a

a, b, c, d = means on the same raw (between treatments) significantly (p≤0.05)

Statistical Analysis

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 and the statistical package for social sciences (SAS, 2010) was used to analyze the data.

RESULTS AND DISCUSSIONS

Table 2 shows the effect of adding crushed laurel leaves to the diet on the immune response (Delayed type hypersensitivity test (DHT), in broiler chickens, where it was noted that the significant superiority (P≤0.05) was to the fourth treatment T4 compared to the other treatments in the traits of cellular immunity antibody titer against Newcastle disease (ELISA), and the relative weight of bursa of Fabricius. It reached (0.243, 2917.2 and 0.124) respectively, while the first treatment (control) recorded the lowest rates and reached (0.168, 2719.6 and 0.065), respectively It was observed a significant superiority (P≤0.05) for the two treatments (T1 and T3) on the first treatment (T1). As for the trait of the bursa of the Fabricius index, the superiority for the fourth treatment (T4) was significant (P≤0.05) compared to the rest of the treatments It reached (1.908). The treatments (T2 and T3) were significantly (P≤0.05) excelled compared to the first treatment (T1control) for the same trait It reached (1.508 and 1.554), while the first treatment was recorded (1,000). All laurel leaves treatments added to the diet gave the highest immune performance compared to the control treatment, because medical plants have a great role in raising the immune response, which stimulates the immune system for birds, where it works to increase the effectiveness of this system by raising the level of antibodies against pathogens (Mohammed 2018, Baytop 1999). As for the significant increase in the relative weight of the bursa of Fabricius and the bursa of Fabricius index in laurel leaves treatments compared to the control treatment, this indicates a significant improvement in the activity of the bursa because it is responsible for humoral immunity in birds, where the bursa vesicles are responsible for the maturation of B cells that are responsible for antibody production (Al-Shiekhli 2003, Fdam et al 2016). The increase in their size indicates the increase in their production of B cells, thus the increase in the production of antibodies, which raise the immune response.

Table 3. Effect of adding crushed laurel leaves to the diet on the logarithmic numbers for the total aerobic bacteria, coliform bacteria, and *Lactobacillus* bacteria in the contents of the duodenum and the ceca for broiler chickens

Treatments	Duodenum			Ceca		
	Total aerobic bacteria	Coliform bacteria	*Lactobacillus* bacteria	Total aerobic bacteria	Coliform bacteria	*Lactobacillus* bacteria
	Mean ±SE					
T1 (control)	0.06 ± 5.27 a	0.13 ± 11.31 a	0.05 ± 3.73 c	0.04 ± 3.97 a	0.08 ± 7.46 a	0.03 ± 2.91 c
T2 (1 g laurel leaves)	0.05 ± 4.61 b	0.11 ± 10.82 b	0.04 ± 4.14 b	0.03 ± 3.41 b	0.07 ± 7.06 b	0.02 ± 3.47 b
T3 (2 g laurel leaves)	0.04 ± 4.57 b	0.09 ± 10.73 b	0.03 ± 4.22 b	0.03 ± 3.35 b	0.07 ± 6.98 b	0.03 ± 3.56 b
T4 (3 g laurel leaves)	0.05 ± 4.35 c	0.09 ± 10.58 c	0.04 ± 4.53 a	0.04 ± 3.16 c	0.05 ± 6.71 c	0.02 ± 3.66 a

a, b, c, = means on the same raw (between treatments) significantly (p≤0.05)

Table 3 shows the effect of adding crushed laurel 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 results indicated that there is a significant decrease in the total numbers for the aerobic bacteria and coliform bacteria in the duodenum and cecum in favor of the fourth treatmentT4 Recorded (4.35 and 10.58 cfu / gr compared to the other treatments. It was showed a significant (P≤0.05) decrease in the total number for aerobic bacteria and Coliform bacteria for the two treatments (T1 and T3) compared to the control treatment (T1) Which recorded the highest number of harmful bacteria (5.27 and 11.31 cfu / gr).. It also shows a significant superiority (P≤0.05) in favor of the fourth treatment (T4) in the number of Lactobacillus bacteria for the two regions of the duodenum and cecum It reached (4.53 and 3.66 cfu / gr) compared to other treatments compared to other treatments. Significant superiority (P≤0.05) continued in the logarithmic number of Lactobacillus in favor of the treatments (T3 and T2) compared to the first treatment (T1control) which recorded the lowest number of beneficial bacteria (3.73 and 2.91 cfu / gr). While there were no significant differences between the two treatments (T2 and T3) 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 laurel 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 laurel leaves contain phenolic compounds that have antibacterial activity against the positive and negative pathogenic bacteria for Cram (Al-Shahat 2000, Bulbul et

al. 2015). 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 (Admas 2016, Erturk et al. 2006).

CONCLUSION

The results in this study indicated that adding laurel leaves to the diet can improve the immune traits response and microbial content for the digestive tract in broiler chickens.

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