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Effect Adding *Petroselinum sativum* L to the Diet of layer Chicken for Reduce Fecal Bacterial Load

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Abstract: The present study was undertaken to assessment adding herbal preparation(*Petroselinum sativum* L) in different series of doses 0.25% , 0.75 % and 0.125 % in chicken of layer feed breed on the total fecal bacterial count, as well as the count of coliform bacteria and the count of lactobacilli bacteria.

Methods: The study was carried out in 35 weeks old Isa Brown breed(72 birds) with 18 birds per group for 3 replicate 6 birds in each. Control group T1 was fed only with the ordinary feeding without any addition of herbs while in the treatment groups T2, T3 and T4, different levels of herbal preparation were added with ordinary feed of birds as mentioned above. The assessment of these experiments was carried out for 21 days and a sample composed of Six birds from each group were selected and the data collected from each bird

Results: All groups showed a significant reduction ($p<0.05$) in both of total fecal bacterial count and faecal coliform count and the differences were more apparent in the T4 as it compared to control group also a significant increment ($p<0.05$) was showed in lactobacilli count in all the groups with more ever increment in T4 when compared with control one.

Conclusion: It can be concluded that the herb *Petroselinum sativum* L supplementation at 0.25% ,0.75% and 0.125 % level to the layer diet causes a definite reduction in the faecal total bacterial count and faecal coliform count and increment of lactobacilli count specially at 0.125% level which recorded best value.

Keywords: *Petroselinum sativum* L , layer chicken, fecal bacterial load.

1.Introduction

Modern intensive poultry production has realize tremendous improvements, consequential in outstanding and economic production of chicken meat and eggs with high quality and safety. The introducing of feed additives in poultry diet was an considerable constituent in improving this achievement. In general most of feed additives mixed with poultry diet contain antioxidants and/or antimicrobials[1].

A numeral of aspects must taken into consideration to preserve the production activity of the birds, specially the integrity of gut which has become the most studied area last times [2]. When the health of gut is sufferer, all activities of digestion and absorption are decline because it is a principal and multifaceted area that involve nutrition on top of physiology, microbiology and immunology. The gut is consist of numeral environmental microorganisms such as bacteria, viruses, protozoa and fungi, however bacterial microorganisms are the prevailing of others [3]. The bacterial colonization in the digestive tract is determined by their own interactive material predilections and the chemical component of the contents [4].

The bacterial diversity and the equivalent of microbes in the digestive tract is affected by several factors as brooding conditions, periods of high challenge, environment, feed and bio security [5], infections by bacteria or viruses [6]. livability of the herd as it impairs the feeding efficiency [7]. To increase validity of the gut,



growth promoters and antibiotic were introduced in feeding of poultry since last fifty years. But the increased risk of using antibiotics and the emergence of strains of resistant organisms prompted the European Union in January 2006 to ban the use of antibiotics [8].

Although, the limitation use of antibiotics may also cause problems in performance [9] while seeking an alternative start. In the present scenario, plant feed additives are important for improving growth performance in animals [10, 11]. Herbs are expected to be considered a safer alternative, such as growth catalysts [12].

The ability of essential oils to improve the performance of poultry growth has been confirmed by its ability to increase the activity of food digestion by encouraging the digestive enzymes to self-excretion and activation, so the microbial ecosystem must be neutralized to achieve health of digestive system.[13]. Parsley (*Petroselinum sativum* L.) is often grown as an annual culinary herb and grows widely in West Asia and Europe [14]. The seeds of this herb have 2-8% of essential oils and the most important is tetramethoxyallylbenzene, as well as other oils such as alpha-pinene, apiol, myristicin which are the basic components of it.

Parsley is also considered the origin of antioxidants and flavonoids, especially apigenin and luteolin as well as origin of vitamin A, vitamin C, vitamin K and folic acid[15].

The results of analysis of dried parsley showed that one gram or about half a tablespoon contains approximately 6.0 micrograms of lycopene, 10.7 micrograms of alpha carotene plus 82.9 micrograms of lutein + zeaxanthin and 80.7 micrograms of beta-carotene [16]. Parsley seeds have been used to eliminate plague and intermittent fever, while exterior appliance of leaves may possibly assist to reduce the spread of tumors. It is also tonic and laxative and an intestinal gas chaser. strong extract by filling the root of a great service in gravel, stones, kidney congestion, jaundice and ascites[17]. *Petroselinum crispum* have shown (inhibition ranging from 7mm to 20 mm) good antibacterial activity versus four micro-organism *E-coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* [18].

Thus, the study was then proposed to estimate the addition of *Petroselinum sativum* L. (parsley) to the diets of layer hen in order to reduce *fecal bacterial count*, *total coliform count* and improve *lactobacilli count*.

2. Materials and Methods

2.1.Location and Birds of experiment

The experiment was accomplished at a cercal of agricultural research farm at Baghdad city with a total of 72 grower birds ISA BROWN breed in 35 weeks old. 18 birds per group (Grower21 days), with 3 replicate ,6 birds in each . The birds were raised in cage with controlled feeding. The experiment was completely randomized design and dietary treatments were conducted in, Table (1). The diets were composition to complete requirement by the National Research Council (NRC) for layer(19) are represented in table(2 and 3)

Table 1 :Dietary treatments

Groups (n=18)	Treatments
T1(Control)	Ordinary feed without any Parsley preparation
T2	Ordinary feed with 0.25% Parsley preparation
T3	Ordinary feed with 0. 75 % Parsley preparation
T4	Ordinary feed with 0.125% Parsley preparation

2.2. Diets of experiment

Table 2. The formulas and calculated nutrient of the basal diet

Ingredient%	Finisher
Total crude protein (%)	19.3
Fiber (%)	2.67
Fat (%)	5.73
Methionine + cystine	1.02
calcium	0.85
phosphors	0.49
methionine	0.68
cystine	0.33
lysine	1.19
Total metabolizable energy (kcal / kg)	3157

Table 3. Chemicals analysis of the experiment diets .

Ingredient%	Finisher
corn	46.22
Soybean meal	27
wheat	20
oil	3.2
Premixe	2.5
Di calcium	0.3
limestone	0.6
methionine	0.18
lysine	-
salt	--
Total weight	100

2.3. preparation of herb (*Petroselinum sativum* L)

The herb (*Petroselinum sativum* L.) was cut by table knife, then it was rinsed, shade dried out and finally crushed to be as a powder. 100 grams of each dried parsley was gotten, mixed-up and filled in tight plastic bottle. (16)

2.4. Collection and preparation of samples

Fecal samples were gathered aseptically from the experimental birds (Six birds from each group). The samples were suspended promptly after collection in 9 mL of sterile normal saline and serially diluted from test tube 1 to test tube 10 and discarded 1 mL from test tube 10. From that 0.1 mL sample is taken in agar plate following spread plate technique and it is incubated at 37 °C for 24 hr (20).

2.5. Evaluation of total bacterial count of feces

The counting medium of agar plate was prepared with a suspension of 23.5 g per 1 liter of distilled water. It was boiled to completely melt. The agar medium was placed in autoclave for sterilization at a temperature of 121 °C for 15 minutes. The medium was then left until temperatures reached about 55 degrees Celsius, after which the media was poured into the plates and incubated for overnight to ensure sterility of the dishes. After that (100 µL) of diluted fecal sample was added to each plate then spread using a sterile swap. The inoculated plates were re-insulated for 24 hrs at 37 °C (21). The colonies on the plate were counted using a colony counter. The bacterial count was carried out at Ministry of scientific and technology /circle of agricultural research lab.

2.6. Evaluation of coliform bacterial count of feces

MacConkey Agar medium was prepared with a suspension of 55.07g in 1L of distilled water. This was applied to the boiling point for dissolution and then sterilized by autoclave at 121 °C for 15 minutes. Later than waited for cooling to about 55 °C, it was flushed into a petri dish and verified sterility through incubation for overnight. A (100 µL) of diluted fecal sample was then extended to the plates. The plates were incubated for 24 hrs at 37 °C (21). The colonies on the plate were counted using a colony counter. The bacterial count was carried out at Ministry of scientific and technology /circle of agricultural research lab.

2.7. Evaluation of the count of lactobacilli

Lactobacillus was counted on the MRS agar. About 55 grams of the *Lactobacilli* MRS broth media was dissolved in (1L) of distilled water and were well mixed then autoclaved at 121 °C for 15 min. The prepared media was cultivated by fecal sample and incubated for 18 to 48 hrs at 35 ± 2°C (21). Results were expressed as log of colony forming units (CFU) per gram of (20).

2.8. Statistical Analysis

By using ANOVA variation analysis, all the data were analyzed. Least significant difference (LSD) was applied among dissimilar groups at the level of 5% (22).

3. Result

The assessment of the *Petroselinum sativum* L preparation on total bacterial count, fecal coliform count and lactobacilli count (Cfu/mL) in the fecal samples of layers is demonstrated in table No.5, and Figure 1. The results showed significant ($P < 0.05$) differences among treatments in the end of studied.

The *fecal total bacterial count* was elevated in the group with only the ordinary feed that represent the control group (5.955 ± 0.207). In contrast to the treatment groups, there are significant ($p < 0.05$) reduction among the other groups. Group 2th, 3th were recorded (5.840 ± 0.238 , 4.866 ± 1.088) respectively. Group 4th recorded significant reduction among all treatment (3.300 ± 0.48).

There were significant ($P = 0.05$) differences among treatments in the end of studied of *total faecal coliform count* load was increased in the control which was fed only with the regular feed as against the treatment groups (5.799 ± 0.301). Group 4th list lower value among all groups of study (3.430 ± 0.650) then followed by groups 3th, 2th were set down (4.087 ± 0.389 , 5.630 ± 0.650) respectively.

While *lactobacilli count* listed significant ($P = 0.05$) differences among treatment in the end of studied. The first group recorded lowest value (6.016 ± 0.062) among all treatment. In the other hands group 4th list best value (8.593 ± 0.447) then followed by groups 3th, 2th (7.443 ± 0.402 , 7.430 ± 0.50) respectively.

Table 4: Effect of *Petroselinum sativum L* on evaluation *total bacterial count*, *fecal coliform count* and *lactobacilli count* (Cfu/mL) in layer chicken, (Mean \pm SE), n=24

Treatments	fecal total bacterial count	<i>fecal coliform count</i>	<i>lactobacilli count</i>
T1	5.955 ± 0.207 a	5.799 ± 0.301 a	6.016 ± 0.062 c
T2	5.840 ± 0.238 a	5.630 ± 0.650 a	7.4305 ± 0.50 b
T3	4.866 ± 1.088 b	4.087 ± 0.389 b	7.443 ± 0.402 b
T4	3.300 ± 0.48 c	3.430 ± 0.650 c	8.593 ± 0.447 a

Different small letters (a, b, c) denoted significant ($p = 0.05$) differences among groups

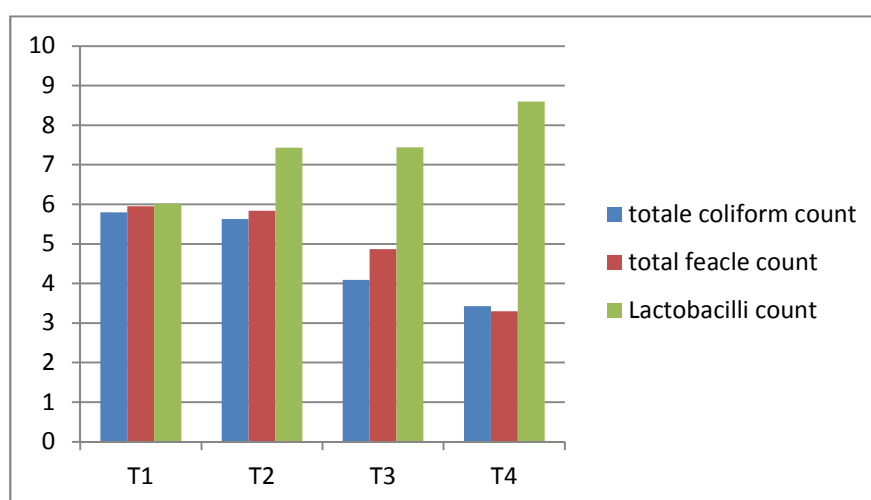


Fig 1: : Effect of *Petroselinum sativum L* on evaluation *total bacterial count*, *fecal coliform count* and *lactobacilli count* (Cfu/mL) in layer chicken, (Mean \pm SE), n=24.

4. Discussion

The results of the present study recorded significant ($P < 0.05$) differences among all treatments specially 4th groups which was recorded significant ($P < 0.05$) decrement of *fecal total bacterial count*, *fecal coliform count* (Cfu/mL)) as compared with other nutrition groups and recorded significant ($P < 0.05$) increment of *lactobacilli count* as compared with other groups. This may be due to have (*Petroselinum sativum* L.) medical compound act as antimicrobial effectiveness against virulent or pathogenic microorganism and this is like to the finding by [23] they examine the antimicrobial activity by using paper disc diffusion method and by micro dilution technique against five pathogenic bacteria (*Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *Yersinia* and *Vibrio cholera*), they found MICs (minimum inhibitory concentrations) of the *Petroselinum Crispum* seeds and leaves essential oil were 8, 0.25% against *S. aureus*, 4, 0.125% against *V. cholera*, 16, 0.5% against *Yersinia enterocolitica* and 32, 1% against the *Salmonella enterica* and *E. coli*, respectively. The results support the high efficacy of *Petroselinum sativum* L to control pathogenic bacteria and use them in the development of new systems to prevent bacterial growth. Also the result of this study similar to the finding by [18] who recorded that *Petroselinum crispum* have shown (inhibition ranging from 20 mm to 7 mm) perfect antibacterial activity against four micro-organism *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. [24] found that the productive qualities was improved in broiler chick if nutrient was supplemented with basil or parsley seeds at (3 g / kg), Also Priya and his colleagues found that a significant decrease ($p < 0.01$) was present in total count of bacteria in feces and coliform bacteria in all the groups that fed on herbs at various levels and the highest decrease was close to one log in group of treatment in contrast to group of control [25]. [26] found the methanol extract of parsley 37 µg/ml dampen the growth of *Micrococcus luteus*, *Staphylococcus epidermidis*, *S. aureus*, *Pseudomonas aeruginosa*,

Escherichia coli, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Candida albicans*, and *Aspergillus niger* using agar diffusion method and explain that Parsley is a medical plant with a variety of proofing pharmacological characteristic containing anti-diabetic, hepato protective, antioxidant, , neuro protective, estrogenic, laxative, anti-ulcer, immunosuppressant, spasmolytic, analgesic, , anti-coagulant, diuretic, hypertensive, antibacterial and antifungal properties.

Housing circumstances, pathogen exposure, and dietary nutrients all play huge affect in modulating the Gut microbiota of poultry [27]. However, the composition of the bacterial communities is referring to be affected mainly by diet, age and gut site [28].

Animal health utility from a stable intestinal microenvironment, for which appropriate development and functioning of the intestinal microbiota and immune system are very important [29].

5. Conclusion

By the findings of this study, it can be concluded that the herb *Petroselinum sativum* L supplementation at 0.25% ,0.75% and 0.125 % level causes a definite reduction in the *faecal total bacterial count* and *faecal coliform count* and increment of *lactobacilli count* specially at 0.125% level which recorded best value. However further studies are needed to assess the effect of the herbal preparations on production performance of layers

6. References

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