Research article

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The effect of garlic and neomycin supplementation in diet on productive and some blood parameters of experimentally infected broiler chickens with *Salmonella typhimurium*

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Abstract

The present study was carried out in order to demonstrate the effect of dietary supplementation of garlic and neomycin in reducing the experimental infection of broilers with *Salmonella typhimurium* & the improvement of productive, physiological parameters all over the duration 42 days. 200 broiler chicks (Hubbard flex) were divided into four groups: T1 (negative control): chicks fed on diet without any addition without *Salmonella* challenge, T2: chicks fed on diet mixed with 5% garlic powder (at 3 day age) plus *Salmonella* challenge, T3: chicks fed on diet with neomycin 0.5% (at 3 day age) plus *Salmonella* challenge, T4 (positive control): chicks fed on diet free of any addition, but challenged with *Salmonella*. The results showed that the addition of garlic powder to the diet of broilers caused significant reducing *S*. *typhimurium* infection resembling to that of neomycin addition both produced significant decreasing (*P* ≤ 0.05) in the number of excreted *Salmonella* in the feces and the mortality rate in the treatments T2 and T3, in comparison with T4. The productive parameters showed significant increasing (*P* ≤ 0.05) in body weight and weight gain in T1, T2, T3 when compared with T4, while the percentage of hemoglobin showed non-significant increasing in T1, T2, T3 when compared with T4, and there are significant increasing in WBC count in T2, T3, T4 when compared with T1, also there are significant decreasing in the three treatments when compared with T4 in L/H ratio, while there was no significant difference in differential WBC between all the treatments. As conclusion, the results of our study had demonstrated the antibacterial activity of garlic and neomycin against the experimental infection with *Salmonella* via enhancement of resistance and immunity besides the improvement of the productive and blood parameters.

Key words: Garlic, neomycin, *Salmonella typhimurium*, broilers.

Introduction:

Salmonellosis is considered as one of the most important zoonotic diseases between human and animals as one of the causes of food poisoning produced by broiler meat (1). Many researchers study this disease in poultry like (2) and (3). *S. typhimurium* consider as one of the serotype which isolated from poultry (4). The researchers showed that the infection of chicks with *Salmonella* could occur during the first week of chicks age and the prevention of this infection during this age might inhibit economic losses which induced by Salmonellosis beside the inhibition of percentage of broiler meat contamination (5). Many therapeutic approaches had been used to inhibit these bacteria such as the use of antibiotics which lead to increase the production and feed consumption sufficiency in addition to control different diseases that
cause high mortality rate in poultry flocks (6). at the same time, the improper or unscientific use of antibiotics could affect the consumers health (7). by the consumption of those poultry products by the human beings can cause health hazards including inhibition of intestinal microflora and generation of resistant strains of microorganism(8). The medicinal plants had been recently used as alternative therapeutic approach for antibiotics, as it considered as important plant source of active ingredients such as flavonoids, glycosides, polyphenols and saponines (9). WHO had recorded that 80% of medicinal plants have therapeutic benefits when used in the form of extract as growth promoters (10), also antibacterial and antifungal (11). The garlic is one of the medicinal plants that consumed by human being as afresh or cooked food with no side effect and its characterized by pungent odor not present in other plants (12). The antibacterial ingredients of garlic including Allin which is one of the derivatives of amino acid (cysteine) which convert to active Allicin with antibacterial effect in addition to other garlic components, phytoicidine and scrodinine which also have antibacterial effect (13). Many researches investigate the medicinal importance in disease recovery (14). The present study aimed to identify the effect of garlic feed additive in comparison to antibiotic neomycin on the productive & blood parameters as prophylactic dose against the experimental infection with Salmonella typhimurium.

Materials and Methods:

The present study had performed at one of poultry halls of medicine college-university of Al-Qadisiyah at a period ranged from 1/10/2014 to 11/11/2014 so that the experimental period had continued for 42 days, two hundred chicks not sexed at age one day belonging to the strain of Hubbard flex had been divided into four treatment groups with two replicated for each treatment (25 chicks in each replicated) as following:

T1: (negative control) the chicks fed on diet without any addition without Salmonella challenge.
T2: chicks fed on diet mixed with 5% garlic powder (at 3 day age to the end of experiment) plus Salmonella Challenge.
T3: chicks fed on diet with neomycin 0.5% (at 3 day to the end of experiment) plus Salmonella Challenge.
T4: (positive control) chicks fed on diet free of any addition but challenged with Salmonella.

The chicks had divided in the hall in the form of pens (2×2) m for each replicate, the hall had been provided with automatic gas incubator to control temperature besides to special programs used for chicks vaccination, the chicks had been fed on diet containing 22.06% crude protein and 3079 Kcal/Kg metabolic energy through (1-21) day and diet containing 19.37% crude protein and 3106Kcal/Kg metabolic energy through (22-42) day (Table1).

Table (1): Composition of standard diets

| Fe Feeds | Ingredients | Starter%(1-21)day | Finisher%(22-42)day |
|----------|-------------|-------------------|---------------------|
| Yellow corn | 48.2 | 58.7 |
| Local wheat | 8 | 7.5 |
| Soybean meal(44%CP) | 28.5 | 20.5 |
| Protein meal | 10 | 10 |
| Vegetable oil | 4 | 2.5 |
| Dicalcium phosphate | 1 | 0.5 |
| salt | 0.3 | 0.3 |
| Total | 100 | 100 |

Calculated chemical analysis

| Metabolic energy(kcal/kg) | 3079 | 3102.6 |
| Crude protein | 22.06 | 19.37 |
| Lysine (%) | 1.21 | 1.03 |
| Methionine +cysteine (%) | 0.82 | 0.75 |
| Crude fiber (%) | 3.54 | 3.2 |
| Calcium (%) | 1.2 | 0.95 |
| Supplement phosphor (%) | 0.44 | 0.42 |

The Kg of protein meal contain (2200 kcal/ME, 40% CP, 8% Fat, 3.5% CF, 5% Ph, 1.2% Methionine, 1.2% Lysine, 25 Ash, 3% Ca, 30 mg Bi, 2500 IU D3, 2% Chlorure, 10000 IU Avit., 300 mg E vit., 12 mg Folic acid, 250 mg B12, 120 mg Pantothenic acid, 400 mg Niacin, 50 mg Bi, 5000 mg Choline chloride, 450 mg Fe, 70 mg Cu.

*According to chemical analysis for feed stuffs (15)
The tested microorganism which is used in this study was *Salmonella typhimurium*, it had been obtained from the unit of zoonotic disease(college of Veterinary medicine) University of Al-Qadisiyah, which was serologically diagnosed at the central laboratory of Ministry of Health. The challenge dose was prepared according to method of (16), five pure colonies of the tested bacteria had been transferred from Brain Heart Infusion Agar (BHA) at temperature 4°C then the colonies were put in flask containing 25ml of nutrient agar after mixing well, 0.1ml of the mixture was taken from the flask mix with 9ml of nutrient Agar in standard bottle at temperature 37°C for two hour. Then the bacterial accounting was done according to method (17). Briefly the bacterial suspension was prepared by mixing one ml of the final nutrient broth with 250ml of distilled water with PH 7.2 So the bacterial concentration was about 0.4×10^5 bacteria/ml which was orally administered at dose 0.3ml/chick at age one day according to method (18) followed by feed additive of garlic and neomycin after 3 days of a curative infection. The neomycin powder 0.5% obtained from Provam Company (Jordan) for Veterinary Drug Production while fresh garlic 5% according to (19) has been per chased from the local market then dried grinded in the form of powder followed by chemical analysis of powder sample at nutrition lab/public health department college of Vet. Med. University of Al Qadisiyah according to (20) as following table:

| Table 2: Chemical analysis of garlic powder |
|---------------------------------------------|
| **substance** | **ratio** |
| Moisture      | 9.3       |
| Protein       | 22.9      |
| Fat            | 3.3       |
| CHO           | 51.2      |
| Fiber         | 7.05      |
| Ash           | 6.25      |

**Parameters:**

Productive parameters: The average of body weight for chicks at one day was calculated (gm/chicks) at the end of each week till the sixth week also the weight gain measured besides the calculation of cumulative feed intake (gm. of consumed food) and feed conversion ratio FCR gm of consumed food/gm. weight gain) also the mortality rate accounted, All these productive parameters measured according to (21). Hematological parameters: blood samples were collected randomly at the end of sixth week for each group (5chicks/replicative) from the brachial vein by using EDTA tubes to prevent blood coagulation for measurement of PCV (22), Hemoglobin level was calculated by conversion it to the complex compound called Cyanomethemoglobin through using of Drabkins reagent (23). Red and white blood cell count were estimated according to method of (24) also the accounted method was used by Neubaur chamber hemocytometer in order to calculate the percentage of heterophilic/lymphocyte cell, Natt and Herrick solution was used for the dilution of these cells in blood of broiler chicks (25). Differential white blood cell count was measured by making a blood smear over clean dry slide which then stained with Right stain and let for 10 minutes followed by washing with tap water and drying to be ready for microscopic examination to determine the percentage of each type of white blood cell (26). Count of *Salmonella typhimurium* that excreted with feces was calculated at age of 7,14,28,35 and 42 days according to (27).

**Statistical Analysis:**

CRD was used for analysis of data, Duncans multiple range tests was used for testing the significant differences among means by using SPSS (28).

**Results:**

**Productive Parameters:**

Table (3) showed the productive parameters of broiler chicks at 6 weeks age,T1,T2,T3
revealed significant difference (P≤0.05) in comparison to T4 (positive control) in the averages of body weight (gm) and the weight gain (gm), the body weight levels were 2760, 2716, 2640, 2520 gm respectively, where the levels of weight gain were 2733, 2712, 2691, 2572 gm respectively. T1, T2, T3 and T4 showed non-significant improvement in the average of food consumption and feed conversion ratio, but T1 has the higher level followed by T2, T3 then T4 for both parameters, the food consumption values were 4560, 4530, 4500, 4430 gm respectively, while the values of FCR were 1.64, 1.67, 1.69, 1.72 gm, also there are decrement in the mortality rate in the first three treatments when compared with T4, the levels were 0.66, 0.66, 3% respectively.

Hematological Parameters:
Table (4) revealed that T1 was significantly different (p≤0.05) from T2, T3, T4 in the red blood cell count, the value of it was 3.3 million cell/ml3 of blood at the end of sixth week, from other hand, T2, T3 were significantly different from T4 which showed the lowest value of RBC count, it was 2.06 million cell/ml3 of blood at the end of sixth week. PCV values were nearly similar to those of RBC count for all treatment groups, so that T1, T2, T3 were significantly different in comparison with T4 in PCV value, its levels were 30, 29, 27, 23% respectively.

Whereas T1, T2, T3 showed non-significant elevation in Hb concentration when compared with T4 (6gm/100ml3). finally the same table also showed the significant elevation of WBC in the treatments that experimentally infected with Salmonella (T2, T3, T4) when compared with T1 and also there are significant decreasing in percentage of heterophil/lymphocytes in T1, T2, T3 in comparison to T4, besides the DWBC that showed non-significant difference (p≤0.05) among the four groups (table 5).

Table (5): The effect of dietary garlic and neomycin in differential WBC of broilers (6week)

|          | Bas | Eos | Mon | Lymph | %Het |
|----------|-----|-----|-----|-------|-----|
| T1       | 1%a | 1%a | 65a | 29a   |     |
| T2       | 1%a | 2%a | 67a | 33a   |     |
| T3       | 1%a | 2%a | 66a | 29a   |     |
| T4       | 1%a | 2%a | 65a | 30a   |     |
| Chi-square value | 0.429 | 0.667 | 0.997 | 0.355 |

letters refers to statistical reading.
*different letters means significant difference at (p≤0.05).
*similar letters means non-significant difference at (p≥0.05)

Bacterial count:
Table (6) showed significant inhibition (p≤0.05) in the count of S. typhimurium which excreted in feces of chicks in T2 and T3 when compared with T4 at the age of 7, 14, 28, 35 and 42 days.

Table (3): The effect of dietary garlic and neomycin in some productive parameters of broilers (6 weeks) Mean ± Standard Error

|          | Body weight(gm) | Weight gain(gm/gm) | Feed intake(gm) | FCR(gm) | Mortality rate |
|----------|-----------------|--------------------|-----------------|---------|----------------|
| T1       | 2760.5±5.77a    | 2733.7±0.11a       | 4560.8±17.32a   | 1.64±0.02a | 0±0a           |
| T2       | 2716.66±31.79b  | 2712.2±58.88a      | 4530.1±57.73a   | 1.67±0.02a | 0.66±0.33a     |
| T3       | 2640.1±57.73b   | 2691.43±0.17a      | 4500.2±34.64a   | 1.69±0.01a | 0.66±0.33a     |
| T4       | 2520.7±5.77c    | 2572.5±58.32b      | 4430.3±57.73a   | 1.72±0.03a | 3±0.57b        |

*letters refers to statistical reading.
*different letters means significant difference at (p≤0.05)
*similar letters means non-significant difference at (p≥0.05)
Table (4): The effect of dietary garlic and neomycin in some blood parameters (6 week) Mean ± Standard Error

|       | RBC (mil/ml³) | PCV%       | WBC(1000cell/ml³) | Hb(gm/100ml blood) | H/L      |
|-------|---------------|------------|-------------------|--------------------|----------|
| T₁    | 3.3±0.17ᵃ     | 30±1.15ᵃ   | 22.85±1.15ᵃ       | 7.3±0.17ᵃ          | 0.23±0.017ᵃ |
| T₂    | 2.6±0.17ᵇ     | 29±0.57ᵃ   | 28.76±0.2ᵇ        | 6.73±0.23ᵃ         | 0.24±0.006ᵃ |
| T₃    | 2.6±0.17ᵇ     | 27±0.57ᵃ   | 28±1.73ᵇ          | 6.45±0.02ᵃ         | 0.24±0.005ᵃ |
| T₄    | 2.06±0.03ᶜ    | 23.0±1.73ᵇ | 27±0.57ᵇ          | 6±0.57ᵃ            | 0.3±0.028ᵇ |

*letters refers to statistical reading.
*different letters means significant difference at (p≤0.05)
*similar letters means non-significant difference at (p≥0.05)

Table (6): The effect of dietary garlic and neomycin in bacterial count of broilers feces (different ages)

|       | 7 days      | 14d        | 21 d       | 28 d        | 35 d       | 45 d       |
|-------|-------------|------------|------------|-------------|------------|------------|
| T₁    | Zeroᵇ      | Zeroᵇ     | Zeroᵇ     | Zeroᵇ      | Zeroᵇ     | Zeroᵇ     |
| T₂    | 40×10⁶ᵇ    | 67×10³ᵇ    | 63×10³ᵇ    | 42×10²ᵇ    | 30×10²ᵇ    | 20×10³ᵇ    |
| T₃    | 43×10⁶ᵇ    | 69×10³ᵇ    | 63×10³ᵇ    | 43×10²ᵇ    | 32×10²ᵇ    | 23×10³ᵇ    |
| T₄    | 49×10⁶ᶜ    | 72×10⁵ᶜ    | 53×10⁴ᶜ    | 45×10³ᶜ    | 35×10³ᶜ    | 30×10²ᶜ    |

*letters refers to statistical reading.
*different letters means significant difference at (p≤0.05)
*similar letters means non-significant difference at (p≥0.05)

Discussion:

**Productive parameters:**

The highly significant of T₁ group in comparison to other groups in the body weight and weight gain could be related to the absence of S. typhimurium infection whereas the highly significant of T₂ group on T₄ in these parameters although their infection with S. typhimurium could be belonging to feed additive of garlic powder which have stimulant effect on digestive system of poultry. So that it can improve the function of organs especially liver leading to increase the secretion of digestive enzyme and finally increasing the food consumption and body weight (29). In addition, the garlic contains number of important factors that have benefit for many biological reactions inside the body because it contain calcium, phosphor, iron and copper (30). On the other hand, the highly significant result of T₃ when compared with T₄ in the body weight &weight gain may be belong to the role of antibiotic neomycin in the inhibition of microorganism which compete the absorption of the nutrient substances when add to the food and its role in growth stimulation in the broilers (31). In addition, the non-significant improvement in food consumption and FCR in groups that fed on garlic powder could be result from the increment of positive stimulation of digestive tract to absorb much more quantities of nutrient substances and increase its capacity to digest proteins, cellulose and fat (32). These effects lead to increase FCR. (33) and (34) had improved that the presence of oils in the medicinal plants may have active effect in improvement of ileum capacity and the digestive tract as general leading to increase the ability of starch, fat digestion resulting in improvement of FCR, while the significant inhibition of results in T₁,T₂,T₃ groups in the mortality rate in comparison to T₄ group could be explained as the chicks in the T₄ group have been experimentally infected with S. typhimurium without treatment with garlic or neomycin so that some medicinal plant including garlic have important role in the bolstering of poultry immunity (35) also prevention and treatment of variety of diseases (36).The researcher (37) showed that the medicinal plant including garlic have
important effect in productive performance when added as a food supplementary in broiler diets. All these causes made garlic as one of the most important agents in treatment of much more diseases.

**Haematological parameters:**

The high significant results of T$_1$ group in RBC count be related to the absence of infection with *S. typhimurium* in this group of chicks, whereas T$_2$ and T$_3$ groups had showed significant elevation of RBC count when compared with T$_4$ group and this could be related to the role of garlic powder and neomycin for two treatment respectively, which are added to the diet in order to improve the body requirements for transporting the nutrients and oxygen to cells because of increasing the metabolic rate of chicks that fed on garlic in diet due to its content of phenolic compounds which help in cellular protection from oxidation distress (38). Results of PCV examination for all the tested groups had similar to that variations in RBC count, PCV is concerned with the numbers of red cells so that any increment in RBC count can lead to increment in PCV (39). Also the non-significant elevation of Hb concentration in first three groups could be directly associated with RBC count. Improvement of these hematological parameters for T$_1$, T$_2$, T$_3$ groups in comparison to T$_4$ group was related to the activity of garlic and neomycin in improvement of poultry health due to their antibacterial effects specially garlic which contain active ingredients can increase the hematomical aspects (40). The results were clearly obvious through the improvement of chicks health although presence of experimental salmonella infection, which could be associated to the antioxidant effect of garlic besides its contents of saccharides, amino acids and vitamins specially Vit. B that play an important role in elevation of Hb concentration(41). The reason of non-significant increasing Hb concentration in T$_2$ group was associated with efficacy of garlic in improvement of digestive tract capability in availing of nutrients leading to enhance the production characters, FCR and the body physiology(42). Improvement of chicks immunity was revealed by significant elevation of WBC count in T$_2$ and T$_3$ groups which could be related to the activity of garlic in development of cellular and humeral immunity in addition to inhibition of harmful bacteria in broiler chicks(43). The significant inhibition of heterophilic/ lymphocyte percentage in first three treatments had given good view about the general health of chicks (44) found that these percentages were the best parameters for examination of chicks status and stress level. Increasing of Heterophilic/ lymphocytes percentages refer to the exposure of chicks to sever stress due to increase corticosteroid level in serum (45). inhibition of this percentage in T$_2$ and T$_3$ groups related to the role of garlic and neomycin respectively which resulted in improvement of general health of chicks. We concluded that the feed additives of garlic had produce enhancement of hematomical aspects of the experimentally infected chicks with Salmonella in comparison to those chicks feeding on diet free from garlic. Those results were agreed with (46) and disagreed with (47). also the improvement of general health of chicks which result from adding of neomycin in food agreed with (48).

**Bacterial count:**

Significant inhibition of *Salmonella* count in feces of broiler chicks in T$_2$ and T$_3$ groups when compared with T$_4$ group, this inhibition was related to the feed additive of garlic and neomycin because of the presence of oils in garlic powder which have antibacterial effect(49). Besides the ability of garlic to improve the body immunity against *Salmonella* due to its component of allicin and Sulphur compound that could inhibit the growth of microorganism so that the garlic is seem to be antibacterial against different species of bacteria(50),(51). demonstrated that feed additive of garlic powder to the diet of broiler chicks leading to bolstering of cellular and humeral immunity besides the significant
inhibition of bacterial count, also garlic powder contain four active ingredients: Diallyl monosulphide, Diallyldisulphide, Diallyl trisulphide and Diallyltetrasulphide which characterized by high antibacterial efficacy acting on destruction of disulphide bond that present in the bacterial cell protein leading to impairment of growing and production of bacteria. On the other hand (34) found that the feed additive of antibiotic to chicks diet could reduce the intestinal microorganism leading to increase resistance of chicks against the bacterial infection. As conclusion, giving of garlic powder and neomycin to T2 and T3 groups cannot prevent infection with S. typhimurium, but play an important role in inhibition of this infection in comparison to T4 group.

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