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: T_1 (negative control):chicks fed on diet without any addition without *Salmonella* challenge,T_2:chicks fed on diet mixed with 5% garlic powder (at 3 day age) plus *Salmonella* challenge,T_3:chicks fed on diet with neomycin 0.5% (at 3 day age) plus *Salmonella* challenge,T_4 (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($P\leq0.05$) in the number of excreted *Salmonella* in the feces and the mortality rate in the treatments T_2 and T_3 in comparison with T_4.The productive parameters showed significant increasing($P\leq0.05$)in body weight and weight gain inT_1,T_2,T_3 when compared withT_4 in spite of no significant differences in total food consumption and feed conversion ratio among the four treatment, on the other hand the blood parameters showed significant increasing($P\leq0.05$) in RBC count and PCV in T_1,T_2,T_3 in comparison with T_4, while the percentage of hemoglobin showed non-significant increasing in T_1,T_2,$T_3$when compared with T_4,and there are significant increasing in WBC count in T_2,T_3,T_4 when compared with T_1, also there are significant decreasing in the three treatments when compared with T_4 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 flavonaides, 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 anti-fungal (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:

T_1:(negative control) the chicks fed on diet without any addition without *Salmonella* challenge.

T_2: chicks fed on diet mixed with 5% garlic powder (at 3 day age to the end of experiment) plus *Salmonella* Challenge.
T_3: chicks fed on diet with neomycin 0.5% (at 3 day to the end of experiment) plus *Salmonella* Challenge.
T_4: (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,3.1%Ph,1.2% Methionine,1.2%Lysine,25Ash,8%Ca,30mgB_1,2500IUD_3,2%Chlore,10000IU Avit.,300 mg E vit.,12 mg Folic acid,250 mg B_{12},120 mg Pantothenic acid,400 mg Niacin,50 mg B_6,5000 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 heamocytometer in order to calculate the percentage of hetrophilic/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.T_1,T_2,T_3 revealed significant difference ($P\leq0.05$) in comparison to T_4 (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.T_1,T_2,T_3 and T_4 showed non-significant ($P\leq0.05$) improvement in the average of food consumption and feed conversion ratio, but T_1 has the higher level followed by T_2,T_3 then T_4 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 T_4 ,the levels were 0,0.66,0.66,3% respectively.

Hematological Parameters:

Table (4) revealed that T_1 was significantly different ($p\leq0.05$) from T_2,T_3,T_4 in the red blood cell count, the value of it was 3.3 million cell/ml^3 of blood at the end of sixth week, from other hand,T_2,T_3 were significantly different from T_4 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 T_1, T2, T3 were significantly different in comparison with T_4 in PCV value, its levels were 30, 29, 27, 23% respectively. Whereas T_1, T2, T3 showed non-significant elevation in Hb concentration when compared with T_4 (6gm/100ml^3).finally the same table also showed the significant elevation of WBC in the treatments that experimentally infected with *Salmonella* (T_2, T3, T4) when compared with T_1 and also there are significant decreasing in percentage of hetrophil/ lymphocytes in T_1, T2, T3 in comparison to T_4, besides the DWBC that showed non-significant difference ($p\leq0.05$) among the four groups (table5).

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

	Bas	Eos	Mon	Lymph	%Het
T_1	1%^{a}	1%^{a}	1^a	65^a	29^a
T_2	1%^{a}	2%^{a}	2^a	67^a	33^a
T_3	1%^{a}	2%^{a}	1^a	66^a	29^a
T_4	1%^{a}	2%^{a}	1^a	65^a	30^a
Chi-square value	0	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 ($p\leq0.05$) in the count of *S. typhimurium* which excreted in feces of chicks inT_2 and T_3 when compared with T_4 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(6weeks) Mean ∓Standard Error

	Body weight(gm)	Weight gain(gm/gm)	Feed intake(gm)	FCR(gm)	Mortality rate
T_1	2760.5∓5.77^a	2733.7∓0.11^a	4560.8∓17.32^a	1.64∓0.02^a	0∓0^a
T_2	2716.66∓31.79^b	2712.2∓58.88^a	4530.1∓57.73^a	1.67∓0.02^a	0.66∓0.33^a
T_3	2640.1∓57.73^b	2691.43∓0.17^a	4500.2∓34.64^a	1.69∓0.01^a	0.66∓0.33^a
T_4	2520.7∓5.77^c	2572.5∓58.32^b	4430.3∓57.73^a	1.72∓0.03^a	3∓0.57^b

***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/ml^3)	PCV%	WBC(1000cell/ml^3)	Hb(gm/100ml blood)	H/L
T_1	3.3∓0.17^a	30∓1.15^a	22.85∓1.15^a	7.3∓0.17^a	0.23∓0.017^a
T_2	2.6∓0.17^b	29∓0.57^a	28.76∓0. 2^b	6.73∓0.23^a	0.24∓0.005^a
T_3	2.6∓0.17^b	27∓0.57^a	28∓1.73^b	6.45∓0.02^a	0.24∓0.005^a
T_4	2.06∓0.03^c	23.0∓1.73^b	27∓0.57^b	6∓0.57^a	0.3∓0.028^b

***letters refers to statistical reading.**
***different letters means significant difference at (p≤0.05)**
***similar letters means non-significant difference at (p0≤.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
T_1	Zero^a	Zero^a	Zero^a	Zero^a	Zero^a	Zero^a
T_2	40× 10$^{6\ b}$	67× 10$^{3\ b}$	63× 10$^{3\ b}$	42× 10$^{2\ b}$	30× 10$^{2\ b}$	20× 10^b
T_3	43× 10$^{6\ b}$	69× 10$^{3\ b}$	63× 10$^{3\ b}$	43× 10$^{2\ b}$	32× 10$^{2\ b}$	23× 10^b
T_4	49× 10$^{6\ c}$	72× 10$^{5\ c}$	53× 10$^{4\ c}$	45× 10$^{3\ c}$	35× 10$^{3\ c}$	30× 10$^{2\ c}$

***letters refers to statistical reading.**
***different letters means significant difference at (≤p0.05)**
***similar letters means non-significant difference at (p≤0.05)**

Discussion:

Productive parameters:

The highly significant of T_1 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 T_2 group on T_4 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 T_3 when compared with T_4 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 T_1,T_2,T_3 groups in the mortality rate in comparison to T_4 group could be explained as the chicks in the T_4 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 hematological 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 hetrophilic/ 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 Hetrophilic/ 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 hematological 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:Dially monosulphide,Diallydisulphide,Dially trisulphide and Diallytetrasulphide 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 T_2 and T_3 groups cannot prevent infection with S. *typhimurium*, but play an important role in inhibition of this infection in comparison to T_4 group.

References:

1-Gast Rk. Paratyphoid infection in"Disease of Poultry.(Calnek B W,Barnes H J,Beard CW, McDougold LR, Saif YM.Eds).(10th edition).Iowa State Univ Press.U.S.A. (1997); PP:97-121.

2-AbdulAziz A, ALTebari A. The effect of cinnamonum aqueous extract on the experimental infection with *S.typhimurium* and productive parameters and some biochemical blood parameters in broilers.J.of Kufa for Vet.Medicine Sci. (2011); 2(2):46-59.

3-Meteab BK. Effect of some feed addative on experimentally infected chickens with locally isolated *Salmonella*. Msc thesis. College of Vet. Medicine. AL-Qadisiyah University.AL-Qadisiyah-IRAQ,(2016).

4-Barri A. Effect of cytosine-phosphate-Guanosine oligodeoxynucleotides(CPG-ODN) on vaccination and immunization of neonatal chickens. Msc thesis. Texas A&M University,(2004).

5-Ebtisam JA. The using of lactobacillus as aprobiotic against the infection with E.coli and *Salmonella* in broiler chicken. MSc thesis. (2001); College of Vet. Medicine. Baghdad University. Baghdad-IRAQ.

6-Nita KP. Introduction to the pharmaceutical Sciences.Lippincott Williams and Wilkins. (2007); pp:301-304.

7-Shareef AM, Jamel ZT, Ronis K. Detection of antibiotic residues in stored Poultry Products. J.of Iraqi Vet.Sciences, 23, Supplement, (2009); (45-48).

8-Tajick MA, Shohreh B. Detection of Antibiotics Residue in Chicken meat using TLC. J.of International Poultry Sci. (2006);5(7):611-612.

9-Tipu LA, Pash TN, Ali Z. Comparative Efficacy of salinomycin Sodium and Neeni Fruit(Aadii acht indica)as feed additive anticoccidals in broilers .J.of Int. Poult.Sci., (2006); 1(4);al-a3.

10-Hermandez FJ, Madridi V, Garcia V, Oregon J, Megias MD. Influence of two plant extractson broiler performance,digestibility and digestive organs size.J of Poult.Sci., (2004); 83:169-174.

11-Saeed S, Tariq P. Antibacterial activities of Emblica officials and coriander against Gram

negative urinary pathogens. J. of Pharm.Sci., (2007); 20(1):32-35.
12-Block E. The organic sulfur chemistry of genus Allium Implication for the organic chemistry of sulfur. Dep. CHEM., State international. (in English).31(a) (1992); 1153-1178.
13-Cavallito CJ, Bailey JH. The anti-bacterial principle of Allium sativa Isolation physiological properties & bacterial action .J.of Am. Chem. Sci., (1950); 66.
14-Woo H. Vegetable extracts as antibacterial preservation &their application in food. PhD thesis. (1973); Florida state University.
15-Natianal Research council. Nutrient requirement of poultry. 9th rev.ed. National Academy press, (1994);Washington,DC,USA.
16-Pivinick H, Blanch fild B, Aoust JY. Prevention of *Salmonella* infection in chicks by pretreatment with fecal cultures from mature chickens (Nurmi cultures).J.of Food protect . (1981); (12);909-916.
17-MacFarlane AS, Peng X, Meissler JJ, Rogers TJ, Geller EB, Adler MW, Collee TK, Marmion JG, Fraser A. Particle Medicine Microbiology, 14th ed. produce by Longman Singapore Publishers Ltd: (1996); 385.
18-Line JE.Baily JS,.Cox NA and Steven NJ. Yeast treatment to reduce *Salmonella & Compylobacter* population associated with broiler chickens subjected to transport stress. J. of Poult.sci., (1997); 76:1227-1231.
19-Gbenga E, Onibi O, Adebowala EA, Fajemisin N, Ayobeji VA. Response of broiler chickens in terms of performance and meat quality to garlic (Allium sativum) supplementation. J. of African Agriculture Res. (2009); 4(5):511-517.
20-AOAC.Method of aralysis. 15thed. Association of official Analytical chemists. (1990); Arlington, VA, USA.
21-Abdulla, MS. Productive Performance and Histological Assessment of Gastro Intestinal Tract Broiler chicks resulting from Two Broiler Breeders and their Reciprocal Crosses. M.Sc thesis. (2007); College of Agriculture. University of Salahaddin. Salahaddin- IRAQ.
22-Arecher RK. Hematological techniques for use on animal. (1965); Oxford Black Publication.
23-Varley HA, Gowenlock H, Bell M. Practical Biochemistry.5th edn. William Heinemann Medical Books Ltd. (1980); London.
24-Natt MP, Herrick CA. Anew blood diluent for counting erythrocytes and leucocytes of chicken .J. of Poultry Sci. (1852); 31:735-738.
25-Muir WI, Bryden WL, Husband AJ. Immunity, Vaccination and the avian intestinal tract. Develop. Compar. Immune. (2000); 24:325-342.
26-Ritchie BW, Harrison JK, Harrison RL. Avian Medicine: Principle and Application. Wingers Publishing, Inc, (1994); Florida.
27-Harrigan WF, McCance MF. Laboratory methods in food and dairy microbiology academic press. (1976); London.
28-SAS. SAS/TAT user's Guide version 6.4th ed. (2001) ;SAS Institute Inc.Gary.NC.
29-Jamros D, Kamal C. Plant extracts enhance broiler performance. In non- ruminant nutrition: Antimicrobial agents and plant extraction immunity, health and performance. J. of Anim.Sci. (2002); 80 (E.Suppl.1):41.
30-Fleischauer TA, Arab L. Garlic and cancer: A critical Review of the Epidemiological Literature. J. of nutrition. (2001); 131:1032S-1040S.
31-AbdulKareem AH. The effect of some antibiotics in the experimental infection with *Mycoplasma* and measure it in serum and muscles in broilers. MSc thesis. (1985); College of Vet. Medicine. Baghdad University. Bhaghdad-IRAQ.
32-Kareem SK. The improvement of resistance and productive performance of broiler chicken against Newcastle and gomboro diseases by Garlic,Nigella Sativa and Foenum-graecum.PhD thesis. (2006); College of Vet. Medicine. Baghdad University. Baghdad-IRAQ.
33-Williams P, Losa R. The use of essential oil &their compounds in poultry Nutrition. J. of World Poultry-Elsevier, (2001); 17:14-15.
34-Sirvydis HV, Robiniene Pirndokiene V, vencius D Phytobiotic add value to broiler feed .J.of World Poultry. (2003); 19(1):16-17.
35-Soltan MA, Shewita RS, katcha MI. Effect of dietary anise seeds supplementation on growth performance, immune response, carcass traits& some blood parameters of broiler chickens. Int.J.of Poult .Sci. (2008); 7 (11):1078-1088.
36-Adibrmoradi M, Navidshad B, Seifdavati J, Royan M. Effect of dietary garlic meal on histological structure of small intestine in broiler chickens.J.of Poultry Sci. (2006); 43: 378-383.
37-Shalaby AM, Khattab YA, Abdel Rahman AM. Effect of garlic and chloramphenicol on growth performance&physiological parameters and survival of nile tilpia (oreochromis niloticus). J. of Venom. Anim. Toxins incl. Trop. Dis. (2006); 12: 172-201.
38-Sturkie PD. Avian Physiology4th ed. Newyork, (1986); Heidelberg Barlin, Springer Verlage.
39-Al-Hasani DH. Poultry Physiology. College of Agriculture. Baghdad University press.(2000); Baghdad.
40-Braun L, Cohen M. Licorice .In: herbs and natural supplements. 2nded. Elsevier Australia (2007); PP: 786-807.
41-Duke J, Bogenschutz-Godwin MJ, Ducellier G, Duke PK. Hand book of Medicinal Herbs .2nd.ed. CRC Press, Inc. Boca Rataon ,F1 .,USA, (2002); PP:461-466.
42-ALBaddy MA, Jalal AK. The effect of dietary supplementing of black seeds, trigonella foenum and garlic powder in productive performance of broilers. J. of Anbar for Agricultural Sci. (2009);7(4).
43-ALHamadany HK. The effect of adding garlic powder to ration in productive, physiological and immunological parameters of broilers. Msc thesis. (2005); College of Agriculture. Baghdad University. Baghdad-IRAQ.

44-ALDaraji HJ. The study of some physiological parameters and heat resistance for broilers and comparison it with commercial hybrids. Msc thesis. (1995); College of Agriculture. Baghdad University.Baghdad-IRAQ.

45-Dosary AW. Study the effect of aquatic extracts of Allium Sativum and Zingiber Officinate on some physiological and biochemical characters of broiler and native Iraqi sheep. J.of Anbar for Vet. Sci. (2012);(5),issue(1).

46-Abuzeed YJ. Evaluation of some properties of immune response in broiler vaccinated with different programs against Newcastle disease. Msc thesis. (2008); College of Vet. Medicine. Duhok University of Kurdistan region-Iraqi.

47-Al-Hayani WK. Effect of garlic powder supplementation to the diet on productive and physiological performance of Hy-line hens.J.of Poultry Sci. (2007);2(3): 134-121.

48-Adel A, Intisar N, Ahmed A. The effect of diet supplementation with antibiotic and Ginger on Broiler performance. J. of Anbar for Agriculture Sci(2010);8(4).

49-Baratta MT, Dorman H, Deans S, Figueiredo A, Barroso J, Ruberto G. Antimicrobial and Antioxidant properties of some commercial essential oils.J of Flav Frag. (1998); 13:235-244.

50-Farbman KS, Barnett ED, Bludue GR, Klein JK. Antibacterial activity of Garlic&onions: Ahistorical perspective. J. of pediatric infectious disease. (1993); 12(7):6123.

51-Lee YL, Cesurio T, Wang Y, Shanbrom E, Thrupp L. Antibacterial activity of Vegetables and juices. J.of Nutrition, (2003); 19:994-996.