Plant Archives Vol. 18 No. 2, 2018 pp. 1337-1340 *ISSN 0972-5210*

USING OF SMASHED *ZINGIBER OFFICINALE* AS A DIET SUPPLEMENTATIONTO INCREASE THE IMMUNE RESPONSE AGAINST *SALMONELLATYPHIMURIUM* INFECTION IN CHICKEN

Bassam G. M. Al-khatib1 and Alan A. Noori[1] and Ihsan Mohammed Shihab[2]

[1]Department of Animal Production, Agricultural Faculty, University of Baghdad, Iraq
[2] Department of Poultry Diseases, Veterinary Faculty, University of Baghdad, Iraq

Abstract

Sixty chickens at age 30 days involved in this study. This chicken divided to three equal groups control, second group give *Salmonella typhimurium* Antigen orally 2×10^9 bacteria /ml only, third group give *Sal.* Antigen and the ginger with the fed concentrate about (95:5) w/w ratio) for six weeks. Each chicken a blood smear was and staid with Wright stain, till differentially read mean H, L and H/L ratio, also study phagocytic activity and delayed hyper sensitivity test.

The study revealed that significant reduction ($p<0.05$) in lymphocytes in group three about (30.13, 35.27) % in the group tow compared with control 58.36%, whereas heterophils recorded significant increase ($p<0.05$) (66.57, 59.25) % respectively in compared with control group 37.48%. H/L ratio revealed significant increase ($p<0.05$) in group three about 3.5 folds while it was only 2.5 folds in the group tow, phagocytic activity revealed a significant difference ($p<0.05$). This activity was maintain for 5, 10, 15 min in group three about 3.53, 3.32, 3.21 respectively but in group tow the activity maintain for 5, 10 min. Highest activity in 5 min in group three 3.53.

CMI measured as relative increase in wattle thickness was higher in the group three only at 24 hr after inculcation of Antigen of *Sal*.

Key words: *Salmonella typhimurium, Zengiber officinale, immune* response, broiler.

Introduction

The immune system has the main importance to protect the body from the negative effects of the pathogenic microorganisms. The system has specialized and non- specialized kinds of immunity. Macrophages, antigen presenting cells (APCs), neutrophils and Natural killer (NK) cells the non- specialized (general) immunity and in case of the microbes passes the primary barrier, the acquired immune response the cellular immunity (comprising humoral and cell mediated Components) which is effective in this case. Many kinds of antigen Ag like (fungi, viruses, bacteria and toxins) shows by antigen presenting cells (APCs) to the CD+4 T cell determines the type of cytokines secreted. T helper cells and B cells give the secondary, immunoglobulin sub type. The T helper cell response manages macrophage activity, and it controls and destroys the pathogenic microorganisms and it helps to activate the cell mediated immunity, from the other side, it effects on immunoglobulin and secretion of the antibodies (Binjamini *et al*. 2000)

Using the plant products in immunity stimulation are considered as an old usage, but the active element recognition was done with these herbal plants since the nineteenth century (Shuwaili *et al*. 2015). Farnsworth (1990) was found that nearly 64% of world population use medicinal plants to avoid the health problems. There are natural components in these plants which have an important role in the defence against the pathogenic calluses, such as flavonoids and hydroxvlated phenols (Oleforuh-Okoleh *et al*. 2014). Ginger is considered from the important medical plants contained the carbohydrate, squatrebinia, and it contains zingberence compound and volatile oils that contain alcohols, monoterpine aldehydes, and its derivatives are many such as ginerols and shogaols

(El deek *et al*. 2002 ; Ahmed *et at*. 2014). This study was aimed to investigate the effect of ginger tubers powder diet supplementation in enhancement of immune response of poultry.

Materials and method

1- Bacteria recognition and isolation

Salmonella typhimurium were gotten from diseases and poultry diseases branch (College of veterinary medicine), and use of this bacteria was due to its importance in poultry and human pathogenic ability. This bacterium is recognized by the national center of Ministry of medicine.

2- Bacterial count and preparation of infection dose

The bacterial reproduction was done on nutrient broth media and counted by the method of Miles and Misra (1984), and it was 1×109 microbes per ml after 18 hours of incubation at 37°C.

The used dose (2×10^9) microbe per bird (with a 900 gram average weight) as a standard dose of infection and stimulation to the immune system as it was mentioned by Williams and Whitemore (1975). It was prepared by taking 2 ml of the original dose (1×10^9) and given to the chickendirectly through the mouth and by using plastic pipette in which it may be pushed to the vesicle individually.

3- Plant sample collection

Some of ginger tubers were brought from Baghdad local markets, then they were grinded by electrical grinder after tubers drying, then they got powder was storied inside clean plastic bags at room temperature till use.

4- Determination the used dose of ginger powder

The added dose amount of ginger powder to the diet at 5% rate (95.5 w/w ratio) was determined according to (Egwurugwu *et al*. 2007).

5) The used chickens in the experiment

Sixty chickens were bought from local markets at one month age; the relative weight of each bird was 980 grams. They were divided randomly into three groups in which each group contained twenty chickens. The first group represented the control (without *Salmonella* bacteria addition) and with ordinary diet, and the second treatment was given the *Salmonella*to each chicken at (2×10^9) microbe per chicken dose individually with ordinary diet feeding, while the third treatment was given the *Salmonella* (2×10^9) microbe per chicken individually with ordinary diet having ginger tubers powder at 5% ratio for 6 weeks.

6- Preparation of blood smears for Lymphocyte L, Heterophil H and H/L ratio calculation

Blood smears were taken from wing vein of chickens after 9 days from *Sal*was given. Adding by taking blood sample and leaving it to dry and it was stained by Wright stain for 4 minutes and then some of distilled water was added over the stain using same volume of stain, and after 4 minutes, it was washed with ordinary water and air dried according to Lucas and Jamroz (1961). The white blood cells were recognized by using the optical microscope and using the oil lens. One hundred white blood cells was counted using method of Campbell (1988) and H/L index from the total of the hundred blood cells for each chicken and the average of these readings was calculated.

7- The phagocytic activity measurement

Method of carbon clearance assay was used according to Lamont (1986) method to measure the average of blood stream purification from the injected carbon molecules. The carbon molecules were given the second group (having *Sal* only) and the third group was having *Sal* and ginger powder, after passage of ten days from giving *Sal* to both of these groups. The carbon molecules (Indian ink) which was centrifuged at 3000 cycles per minute for 30 minutes, and then the floating part of it represented as a source of carbon molecules which was given to chickens. These carbon molecules were used in 1 ml per one kilogram chicken weight dose and they were injected inside the right wingvesicles, 100 micro liter blood sample was taken before and after passage of 5, 10 and 15 minutes for each taken sample, then the samples were transferred directly in clean tubes which contain 2 ml of 1% sodium citrate and the samples were measured using electronic spectrophotometer at 675 nm wave length after collecting the samples directly.

8- Delayed hyper sensitivity test

This test is considered from the valuable tests in measuring the cellular immunity. The complete microbial antigen of *Sal typhimurium* microbes was prepared by using Lee *et al*. (1981) method in which 1000 mg dose was injected with 0.1 ml of the sterilized physiological solution as best dose may be given a clear reaction in epidermis of one of the wattle of chicken (the right), and the epidermis of the left wattle was injected by 0.1 ml of the physiological solution only and it was considered as a control. This test was done on the sense chicken on the same microbial antigen of the second and third groups only and of the 42 day passage from giving the microbe *Sal* to the chickens. Many studies mentioned that the best delayed wattle reactivity was at 6, 9 and 12 weeks and it may reach to 15 weeks (Lee *et al*. 1981; Al-Murrani *et al*. 1995). The wattles and ear piercing was soft, warm

and losing. Wattles thickness was measured after 24 and 48 hours from test start by using (Al- warnia) and the readings of both of the injected wattles by the complete microbes' antigen and the physiological solution. The relative thickness increase was providing as a measurement of the cellular immunity amount by which is calculated using the following equation that put by Al-Murrani *et al*. (1995).

$$T_r = \frac{T_R - T_y}{T_y}$$

T_r = the relatives increase of wattle thickness

T_R = thickness of the right wattle after injection by the antigen.

T_r = thickness of the left wattle after injection by the sterilized physiological solution

Statistical analysis

Data were statistically analyzed using statistical analysis system program – SAS (2012) to study the effect of ginger supplementation in various traits and compared the significant differences between the averages using the Duncan test (1955) polynomial.

Results and discussion

Table (1) shows percentage means of the H, L cells and H/L ratio of each group given *salmonella* only and the group got *Sal* and ginger powder together and the control group. There was significant decline ($p>0.05$) of L cells after given *Sal* and also after given Sal with ginger powder, and the decline was bigger after giving Sal at 35.27% ratio, while in the group that given *Sal* and ginger tuber powder together, the decline value was 30.13% compared with the control (58.36%), while the H cells increased significantly ($p<0.05$) at both of the last two groups compared with the control treatment and its increase in the first group was 59.25 and in the second group was 66.57 compared with the control (37.48). The total number of white blood cell in the blood plasma showed a greater increase in the *Salmonella* and the ginger group because the stimulatory effect of ginger powder on the immune system Tavakol *et al*. (2015). The lymphatic cells ratio decline may be due either to cells redistribution outside blood stream and they're going to the secondary lymphatic tissues or the destruction of these cells Mashaly and Trout (1994).

Increase of H cells in both of the two groups compere to control may be attributed to their essential and quick role in natural resistance stimulation against the bacterial infection in poultry Powell (1987) and to their early work in body defence to their ability to devour a wide range of microbes, stop the growth and reproduction block till they got body cell mediated immunities later (Shuwaili *et al*., 2015; Barry, 1998). This was followed by increase of H/L ratio average after *Sal*given to the first group and it reached twice times and a half, while in the second group, it was nearly 3 times and half compared with the control, this indicates to the gingers powder ability in speed the immunity response by different ways and those indicated by Benny and vanitha (2004) while the ginger of material present in *Zingier officinal* tubers works on activation of immunity when any pathogenic antigen inter to the body through stimulation of in terleuk in IL6, than effect to B cells and the cellular immunity specially T helper cells.

Table (2) shows absence of statically, important

Table 1: The percentages of L, H cells and H/L ratios in chicken before and after giving *Salmonella* and *Salmonella* with ginger powder.

Treatment	Control	*Salmonella*	Ginger powder + *Salmonella*
L	A 58.36±1.41	B 35.27±1.27	C 30.13±1.32
H	A 37.48±1.36	B 59.25 ±1.54	C 66.57±1.09
H/L	A 0.65±0.04	B 1.67±0.17	C 2.21±0.12

The differences between means with different letters are statistically significant $p<0.05$

Table 2: The rate of the large phagocyte cells (Macrophage) in the chicken before and after giving *Salmonella* and *Salmonella* with ginger powder.

Treatment	Timing (minutes)			
	0	**5**	**10**	**15**
Salmonella	A 2.68±0.03	B 3.01±0.13	B 2.91±0.07	A 2.66±0.02
Ginger powder + *Salmonella*	A 2.74±0.07	B 3.53±0.17	B 3.32±0.09	A 3.21±0.08
	N.S	N.S	N.S	S.D

The difference between the means with different letters is statistically significant by $p<0.05$ in horizontal comparisons, N.S = There is no statistically significant difference in vertical comparisons.

Table 3: The relative increase in the thickness of the wattle in chickens after *Salmonella*and *Salmonella* with ginger powder is given.

Treatment	Relative thickness of wattle	
	48 Hours	**24 Hours**
Salmonella	A 1.86±0.13	A 1.57±0.19
Gingers powder + *Salmonella*	B 2.18±0.12	A 1.56±0.16

The differences between means with different letters are statistically significant $p<0.05$

differences between the first and second groups at all of the time 5,10 and 15 minutes except in time 15 min. in which there was important significant difference ($p<0.05$).

There was an increase in the phagocytic ability at 5 minute timing in the first group (having *Sal* only) and continued till the 10 minutes and then the ability decreased at 15 minutes and reached 3.01, 2.91 and 2.66 respectively. In the second group, the phagocytic ability continued to rise after 5 minutes and at the same rate in 10 and 15 minutes and their values reached 3.53, 3.32 and 3.21 respectively, and they were higher than their values in the first group in all the used times. This may be attributed to gingers powder ability to raise the phagocytic ability level and this was referred by Chang (1995) who indicated that use ginger powder works on increase of mononuclear blood cells stimulation in the blood stream for period last from 18-24 hours. It was also showed by Benny and Vanitha (2004) the ginger powder ability to increase the activity of the phagocyte cells and stimulation of the humeral and cellular immunity.

Table (3) refers to the delayed wattle reactivity test in chickens and the relative wattle thickness at 24 hours was 1.86 in the first group (given *Sal* alone) compared with the second group in which its value reached 2.18, the difference was statically important, but after 48 hours, there was a decline in wattle thickness in the first and second group and the readings were similar. The high increase in wattles thickness after 24 hours in the second group supports the ginger powder role in immunity level rise and the continuity of the cellular immunity activity till 42 days of giving *Sal*. This was mentioned by Benny and Vanitha (2004) who pointed to that ginger's extract ability in cellular immunity specially T helper cells stimulation and stimulate of cytokine secretion for body defense against the bacteria, fungus and viruses infections.

References

Ahmed, M.H., K.A. Abdel, H.E. Malik, K.M. Elamin and B.M. Dousa (2014). Ginger (*Zingiber Officinale*) root powder as natural feed additive for broiler chicks. *Global Journal of Animal Scientific Research*, **2**: 383-389.

Al-Murrani, W.K., H. Hameed, Z.G. Abdul-Gani and A.O. Omran (1995). Some aspects of genetic resistance to *S. typhimurium* in native and white-leghorn chickens. *Dirasat*, **22(2)**:(1995). Jordan.

Barry, G.H. (1998). Avian heterophils in inflammation and disease resistance. *Poultry Science,* **77**: 972-977.

Benjamini, E., R. Coico and G. Sunshine (2000). Immunology, a short course (4th edition), wiley-Liss: Canada, U.S.

Benny, K., H. Tan and J. Vanitha. (2004). Immuno-modulatory and antimicrobial effect of some traditional Chinese medicinal herbs: Areview. *Current Medical Chemistry*, **11**: 1423-1430.

Campbell, T.W. (1988). Avian hematology and cytology. Ist ed. Lowa State Univer. Press. USA.

Chang, C.P. (1995). The effect of Chinese medicinal herba *zingiberis rizhoma* extracton cytokine secretion by human peripherical blood mononuclear cells. *Journal of Ethnopharmacology,* **48**: 13-19.

Egwurugwu, J.A., C.S. Ufearo, O.C. Abanobi and C.R. Nwokocha (2007). Effects of ginger (*zingiber officinale*) on cadmium toxicity. *African Journal of Biotechnology,* **6(18)**: 2078-2082.

El-Deek, A.A., Y.A. Attia, M. Maysa and M. Hannfy (2002). Effect of anise (*Pimpinella anisum*), ginger (*Zingiber officinale* Roscoe) and fennel (*Foeniculum vulgare*) and their mixture on performance of broilers. *Archiv. für. Geflügelkunde*, **67**: 92–96.

Farnsworth, N.R. (1990). The role of ethnopharmacology in drug development, p. 2-11. In: D.J. Chadwick and J. Marsh (Eds). Bioactive compounds from plants. Ciba foundation symposium 154. Wiley, Chichester, U.K.

Lamont, S.J. (1986). Genetic associations of the recticuloendothelial activity in chicken. Proc. 3rd world Congress on Genet. App. to livestock prod. Agriculture Communication, Univer of Nebrasha, Lincoln, NB. Vol. XI: 643-647.

Lee, G.M., G.D.F. Jackson and G.N. Coopers (1981). The role of serum and biliary antibodies and cell mediated immunity in the clearance of *S. typhimurium* from chickens. Vet. *Immunology and immunopathology*, **2**: 233-252.

Lucas, A.M and C. Jamroz (1961). Atlas of avian hematology, US. Dept. *Agri, Washington*, D.C.

Mashaly, M.M. and J.M. Trout (1994). The effect of adrenocorticotropic hormone and heat stress on the distribution of lymphocyte population in immature male chickens. *Poultry Science*, **73**: 1694-1698.

Miles, A.A and S.A. Misra (1983). The estimation of bactericidal power of the blood. *The Journal of Hygiene*, **38**: 732-739.

Oleforuh-Okoleh, V.U., G.C. Chukwu and A.I. Adeolu (2014). Effect of ground ginger and garlic on the growth performance, carcass quality and economics of production of broiler chickens. *Global Journal of Biochemistry and Biotechnology*, **3**: 225-229.

Powell, P.C. (1987). Macrophages and other nonlymphoid cells contributing to immunity in avian immunology Basis and practice. **1** : 195-212.

SAS (2012). Statistical Analysis System ,User's Guide. Statistical .Version 9. 1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Shuwaili, M.A., E.I. Ibrahim and A M.T. Naqi (2015). Effect of dietary herbal plants supplement in turkey diet on performance and some blood biochemical parameters. *Global Journal of Biochemistry and Biotechnology*, **4**: 153-157.

Tavakol, M., H. Khodaeiand J. Nasr (2015). Ginger powder effect on the immune system in broilers exposed to Salmonella infection. *Der. Pharma Chemica*, **7(4)**:266-269.

Williams, J.E. and A.D. Whitemore (1975). Influence of age on serological response of chicken to *S. typhimurium* infection. *Avian Diseases,* **19(4)**: 475-516.