IMPACT OF GINGER POWDER SUPPLEMENTATION IN BROILER DIET ON THE IMMUNE STATUE DEVELOPMENT AND SMALL INTESTINE MORPHOLOGY

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ABSTRACT

A study using 300 one-day-old Ross-308 broilers was conducted to assess the effects of dried ginger root powder (*Zingiber officinale*) addition at percentages (0.2, 0.4 and 0.6) % in the treatments T1, T2 and T3 respectively on growth performance, immune status and small intestine morphology of broiler chickens at different ages. Compare with the control group (T0) without adding ginger powder.

The broiler body weight, body weight gain, viability, production index, and economic profit impacted with addition of ginger powder significantly (P≤0.05) increase in T3 at ages (1-21 d) and (36-42). As well as feed conversion ratio (FCR) particularly improved. In addition the morphological of small intestine goblet cells number in (duodenum , jejunum, and ileum), immunological delayed type of hypersensitivity DTH and ELISA titer against Newcastle (ND), Gumboro (IBD) and Infectious bronchitis (IB) diseases of broiler chickens were significantly higher (P≤0.05) at the ages 21, 35 and 42 d. While mortality and H/L ratio were significantly lower in the treatments of ginger supplementation especially in T3 compare with the control group without ginger addition.

INTRODUCTION

Feed additives are substantive supplements for improving the utilization of nutrition, immunity and health of animals [1]. In bygone, antibiotics were cosmically utilized as a growth

promoter in animal nutrition [2]. Over the years, feed additives has been fast growing broilers and their short generation period used as sub-therapeutic doses in animal feeds to improve the quality of the product. The essential compounds of ginger evaluated as natural alternatives to feed antibiotics in broiler diets [3].

Ginger (*Zingiber officinale*) is widely used in many countries as appetizer food and as a medicinal herb. The main important protein compounds in Ginger are gingerol, gingerdiol and gingerdione which have the ability to stimulate digestive enzymes affect the microbial activity and having antioxidative activity when used in broiler diets [4]. Ginger supplementation act as an antioxidant broiler chickens blood serum [5]. Many studies have been done to improve the efficiency of poultry products. Considering the importance of broiler growth, well development of gut is necessary to make optimal use of feeds and to provide suitable statues to substantiate the genetic potential of birds [6].

Ginger is a rhizome plant which belonging to Zingibeaceae family. It has medicinal properties against digestive disorders, ginger extract possesses antioxidative features, since it can scavenge superoxide anion and free radicals [7]. Other benefits of these compounds include control of zoonotic pathogens such as *Salmonella, Campylobacter, Escherichia coli* and Enterococci species in the gut [8]. Ginger (Zingiber officinale Roscoe, Zingiberaceae) rhizome is widely used as medical treatment for certain diseases for it contains several compounds that possess strong antioxidant activity. However there is no information on the effect of ginger or its compounds on animal performance and serum metabolites [9].

The aim of this study was to evaluate the beneficial of ginger powder supplementation in broiler diet on body performance, immune statue development and small intestine morphology at deferent ages.

MATERIALS AND METHODS

Experimental Design

The study was carried out in the poultry farm hall in Grdaradha/Animal Resources Dept./College of Agricultural/University of Salahaddin-Erbil/Iraq. A total of 300 one-day-old Ross-308 unsexed broilers chicks were weighed and randomly allocated to four treatments with three replicates of 25 birds, at average starting body weight of (42.10 g) across the groups. Birds were reared in floor pens of identical size (2 × 2) m using wood shaving as litter. The rearing hall

was controlled at standard conditions. The relative humidity was between (65- 75) % and of 25-27 ^{O}C temperature and ventilation and maintained at 20-h light regime 24-h in first week and then (2 Darkness: 10 Lighten: 2 Darkness: 10 Lighten) throughout the trial period till marketing age. The birds were fed with one of six diets: Basal diets T0 (control) and the experimental diets supplemented with ginger (0.2, 0.4 and 0.6) % in the treatments T1, T2 and T3 at 42 days. The ginger was mixed with basal diet manually in each supplemented groups. Feed and fresh water were available *ad libitum*, the feed prepared by Kosar company that contains (3000, 3100, 3175, 3200 kcal/kg) metabolized energy, (23, 21, 20, 19 %) crude protein (starter (1-11d), grower (12-25 d), finisher 1 (26-40 d) and finisher 2 (41-42 d) diet respectively. Birds were vaccinated using combined Newcastle disease virus (NDV) and infectious bronchitis virus on day 7 via coarse spray and drinking water administration and on day 21 through fine spray administration. In addition, birds were vaccinated with IBD at age 5 and 14 d in drinking water.

Body performance measurements

Body weights were measured by digital balance and mortality recorded every day then obtained the average at the ages (1, 21, 35 and 42). In addition the formulas for calculating percentage mortality percentages in every by given the total number of dead birds in a group in every age. So the viability (the ability to live) percentage by: 100 – mortality %.

furthermore, the broiler production Index (BPI) was also computed:

$$BPI = \frac{\text{Body weight (kg)} \times \text{viability (\%)}}{\text{Age (days)} \times \text{FCR (g feed/g gain)} \times 10}$$

Immune statue measurements

In the same ages blood was also collected in tubes from the jugular vein of 12 birds from each treatment. Then serum separated by centrifuge for measuring antibody titer by direct ELISA (Biochek – ELX 800) test for Newcastle disease (ND) Gamboro (IBD) and infectious bronchitis disease (IBV) by using kits from Synbiotics. Delayed type of hypersensitivity (DTH) for Newcastle disease was determined by injecting 0.1 ml antibody intradermal of right wattle, while the left wattle inject by 0.1 ml sterilized functional salt solution which treated with 0.1%

formalin as control group. After 24 and 48 h the thickness of wattles at ages 35 and 42 d, which measured by vernier [10] as in this equation:

DTH= Thickness of right wattle - Thickness of left wattle/Thickness of left wattle

Leukocytes determination

Differential leukocyte count (heterophil and lymphocyte) made on slides stained with Wright-Giemsa and observed in an optical microscope (100x) to determined H/L ratio [11].

Small intestine morphological examination

Intestinal samples were dehydrated, cleared, and embedded in paraffin. Serial sections were cut at 5 µm and placed on glass slides. For all assays, sections were deparaffinized in xylene and rehydrated in a graded alcohol series. Sections were examined by light microscopy [12], [13].

Statistical Analysis

All data were analyzed using CRD (Complete Randomize Design) by SAS [14], as per variance. Significant differences among treatment means were determined by Duncan's multiple range tests [15].

RESULTS

In the current study, the results of broiler body weight (g) were significantly ($P \leq 0.05$) higher in the treatment T3 (0.6 % ginger powder) at ages (1-21 d) and in all treatments of ginger powder addition at ages (1-35 and 1-42) days compared with the control (T0: standard diet without adding ginger powder) (Table 1). In addition, the body weight gain (g/bird) and feed conversion ration (FCR: g feed/ g BWG) were significantly ($P \leq 0.05$) improved in the treatments T2 (0.4 %) and T3 (0.6 %) ginger powder at age (1-21) and (1-42) days, thus the birds in all treatments of ginger powder addition were improved BWG and FCR at age (1-35) day compared with the control (T0). While there was no significant difference among all the treatments and at all ages of the study in feed intake (FI) (g).

Table 1. The impact of Ginger powder supplementation in broiler diet influenced on body performances at different ages.

Traits	Age day	Treatments				MSE
		T0	T1	T2	T3	
Body weight (g)	Initial	42.1				
	1-21	804.2 ^{b}	835.0 ^{ab}	872.7 ^{ab}	925.9 ^{a}	45.1
	1-35	1866.9 ^{c}	1973.3 ^{b}	2133.3 ^{ab}	2216.0 ^{a}	142.7
	1-42	2671.1 ^{c}	2808.3 ^{b}	3006.0 ^{ab}	3141.9 ^{a}	163.7
FI (g)/bird	1-21	1211.7 ^{a}	1205.2 ^{a}	1204.4 ^{a}	1219.6 ^{a}	122
	1-35	3248.1 ^{a}	3283.0 ^{a}	3262.3 ^{a}	3260.9 ^{a}	214
	1-42	4995.1 ^{a}	5034.5 ^{a}	5009.0 ^{a}	5052.7 ^{a}	310
BWG (g)/bird	1-21	762.1 ^{c}	792.9 ^{bc}	830.6 ^{b}	883.8 ^{a}	65.3
	1-35	1824.8 ^{c}	1931.2 ^{b}	2091.2 ^{b}	2173.9 ^{a}	147.5
	1-42	2629.0 ^{c}	2766.2 ^{bc}	2963.9 ^{b}	3099.8 ^{a}	176.9
FCR (g feed/ g BWG)	1-21	1.59 ^{c}	1.52 ^{bc}	1.45 ^{b}	1.38 ^{a}	0.065
	1-35	1.78 ^{c}	1.70 ^{b}	1.56 ^{ab}	1.50 ^{a}	0.059
	1-42	1.90 ^{b}	1.82 ^{b}	1.69 ^{a}	1.63 ^{a}	0.084

T0= Control, T1=0.2% ginger powder, T2=0.4% ginger powder , T3= 0.6% ginger powder. The same superscripts within rows means non-significant. FCR: feed conversion ratio. $^{a-c}$ Means within rows with different superscripts differ significantly at ($P \leq 0.05$).

The effects of dietary ginger powder addition on broiler viability percentage were significantly ($P \leq 0.05$) higher in the treatment T3 (0.6 %) at age (1-21) day, and in the treatments T2 and T3 at age (1-35) day (Table 2). In addition, there was high significance in all the treatments of ginger addition at age (1-42) day. Furthermore, broiler production index (BPI) was raised significantly ($P \leq 0.05$) in all the treatments of ginger addition at different ages. While, mortality percentage was significantly ($P \leq 0.05$) lower in the treatments T2 and T3 at age (1-21) day and in all the treatments of ginger addition at ages (1-35) and (1-42) days.

With regard to age effect, the mortality percentage recorded significantly ($P \leq 0.05$) lower at ages (1-21) and (1-35) days compared with (1-42) day. While, there was elevate in viability % at ages (1-21) and (1-35) days, so broiler production index (BPI) was elevated at ages (1-35) and (1-42) days which means more economic profit during marketing.

Table 2: The impact of Ginger powder supplementation in broiler diet on the mortality, viability percentages, and broiler production index (BPI) at different ages.

Traits	Age day	Treatments				MSE	Age effect
		T0	T1	T2	T3		
Mortality %	1-21	4.00 ^{a}	3.33 ^{a}	1.33 ^{b}	0.00 ^{b}	0.13	2.17 ^{b}
	1-35	5.33 ^{a}	2.33 ^{b}	1.34 ^{b}	1.33 ^{b}	0.103	2.58 ^{b}
	1-42	9.33 ^{a}	5.66 ^{b}	2.67 ^{c}	1.33 ^{c}	0.245	4.75 ^{a}
Viability %	1-21	96.00 ^{b}	96.67 ^{b}	98.67 ^{ab}	100.0 ^{a}	0.00	97.84 ^{a}
	1-35	94.67 ^{b}	97.67 ^{ab}	98.66 ^{a}	98.67 ^{a}	1.25	97.42 ^{a}
	1-42	90.67 ^{c}	94.34 ^{b}	97.33 ^{ab}	98.67 ^{a}	2.55	95.25 ^{b}
BPI	1-21	231.22 ^{b}	252.88 ^{b}	282.79 ^{ab}	319.50 ^{a}	27.4	271.60 ^{b}
	1-35	283.69 ^{c}	323.92 ^{b}	385.48 ^{ab}	416.48 ^{a}	33.5	352.39 ^{a}
	1-42	303.49 ^{c}	346.59 ^{b}	412.19 ^{ab}	452.84 ^{a}	39.4	378.78 ^{a}

T0= Control, T1=0.2% ginger powder, T2=0.4% ginger powder , T3= 0.6% ginger powder. $^{a-c}$ Means within rows with different superscripts differ significantly at ($P \leq 0.05$).

The ratio of heterophil to lymphocyte means the stress statues of broiler chicken. The results of H/L ratio revealed significantly ($P \leq 0.05$) reduction or improved in the treatments T2 (0.4) % and T3 (0.6) % ginger at the ages 21, 35 day and at the age 42 in all treatments of ginger addition compared with the control (basal diet) (Table 3). The ages 35 and 42 days revealed more effect in H/L ratio as compared with the age 21 days.

Table 3: Effect of ginger powder supplementation on H/L ratio of broiler at different ages.

Age day	Treatments				MSE	Effect of age
	T0	T1	T2	T3		
1	1.075					
21	0.683 ^{a}	0.517 ^{ab}	0.455 ^{b}	0.402 ^{b}	0.103	0.514 ^{a}
35	0.493 ^{a}	0.344 ^{ab}	0.296 ^{b}	0.250 ^{b}	0.0712	0.345 ^{b}
42	0.478 ^{a}	0.279 ^{b}	0.238 ^{b}	0.218 ^{b}	0.316	0.303 ^{b}

T0= Control, T1=0.2% ginger powder, T2=0.4% ginger powder , T3= 0.6% ginger powder . $^{a-c}$ Means within rows with different superscripts differ significantly at ($P \leq 0.05$).

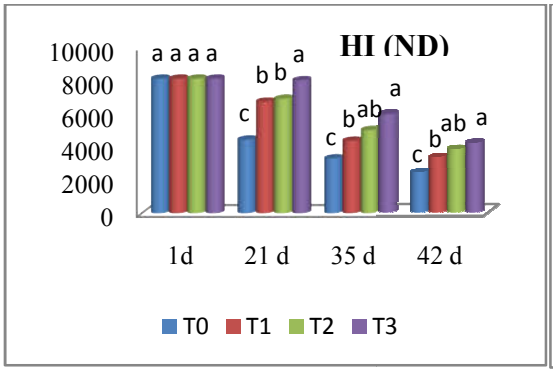
The delayed type of hypersensitivity (DTH) was significantly (P≤0.05) higher at 24 and 48 h after injecting antibodies in the right wattle in both ages 35 and 42 days (Table 4). While, there were no significant differences between the age 35 and 42 d in this regard.

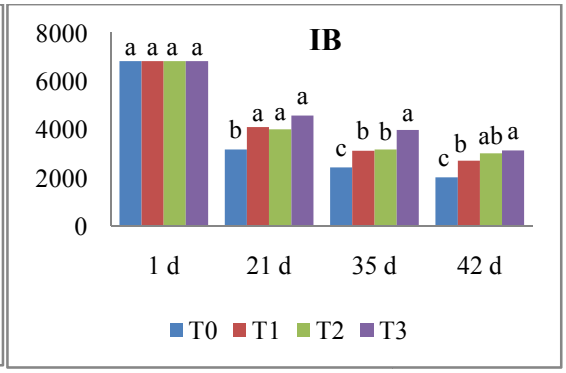
Table 4: Effect of ginger powder supplementation on delayed type of hypersensitivity (DTH) for Newcastle disease (ND) at 24 and 48 h of broiler at ages 35 and 42 d.

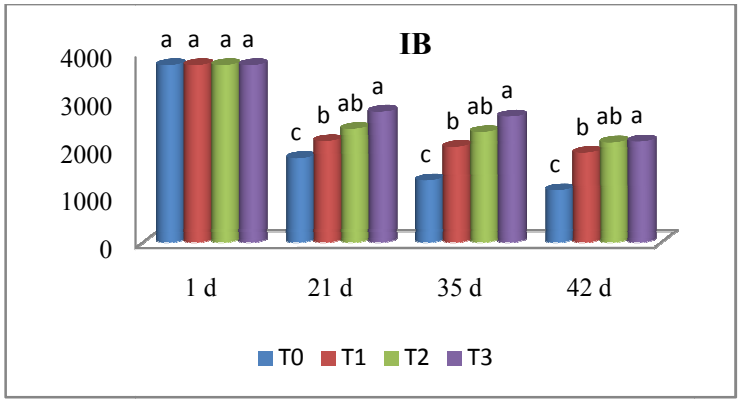
Age		Treatments					Effect
day	Time	T0	T1	T2	T3	MSE	of age
35	24	0.132 ^{c}	0.308 ^{b}	0.344 ^{ab}	0.427 ^{a}	0.037	0.303 ^{a}
	48	0.259 ^{c}	0.433 ^{b}	0.440 ^{b}	0.494 ^{a}	0.035	0.355 ^{a}
42	24	0.125 ^{c}	0.271 ^{b}	0.309 ^{ab}	0.386 ^{a}	0.028	0.273 ^{a}
	48	0.238 ^{c}	0.390 ^{b}	0.392 ^{ab}	0.415 ^{a}	0.029	0.3.16 ^{a}

T0= Control, T1=0.2% ginger powder, T2=0.4% ginger powder , T3= 0.6% ginger powder, DTH: Delayed type of hypersensitivity. $^{a-c}$ Means within rows with different superscripts differ significantly (P≤ 0.05).

The results of using different levels of ginger on immunological ELISA titer against some vaccines of broiler chickens are shown in figures (1, 2 & 3). All the treatments of ginger powder addition were significantly higher (P≤0.05) in Newcastle (ND), Gumboro (IBD), and Infectious bronchitis (IBV) diseases compared with the control (T0) at the ages 21, 35 and 42. Both ages 21 and 35 revealed improvement in ND, IBD and IBV compared with the age 42.







Figures 1, 2 & 3: Effect of ginger powder supplementation on cellular immunity ELISA titer (ng/ml) against HI (ND), Gumboro disease (IBD) and IBV of broiler at different ages.

T0= Control, T1=0.2% ginger powder, T2=0.4%% ginger powder , T3= 0.6%% ginger powder, ND= Newcastle disease, IBD = Gumboro disease and IBV= Infectious bronchitis disease. $^{a-c}$ Means within rows with different superscripts differ significantly ($P \leq 0.05$).

The morphology of small intestine also affected by ginger addition at levels 0.2, 0.4 and 0.6 respectively (Table 5). The results of small intestine length to body weight was significantly ($P \leq 0.05$) higher in the treatment T3 at age 35 and in the treatments T2 and T3 at age 42. In addition, the results of goblet cells number were significantly ($P \leq 0.05$) increased in all the treatments of ginger addition of broiler duodenum at 35 and 42 days, thus the results revealed more number of goblet cells in the treatments T2 and T3 at age 35 and all the treatments at age 42 of jejunum. Furthermore, ileum goblet cells revealed high number in all the treatments of

ginger addition at ages 35 and 42 days compared with the control T0 (without ginger addition). About the effect of age, the broiler small intestine length, the goblet cells number in the duodenum and ileum weren't revealed any differences between the ages 35 and 42 days. While. the age 42 days revealed significantly (P≤0.05) higher in goblet cells number of broiler jejunum compared with the age 35 days.

Table 5: Effect of ginger powder supplementation on small intestine relative length (%) and goblet cells number in duodenum, jejunum, and ileum of broiler at ages 35 and 42 d.

Traits		Age d	Treatments T0	T1	T2	T3	MSE	Effect of age
Small intestine length (cm):BW (g)		35	6.04 ^{b}	6.45 ^{ab}	6.86 ^{ab}	7.25 ^{a}	0.450	6.65 ^{a}
		42	6.55 ^{c}	6.78 ^{bc}	7.02 ^{b}	7.83 ^{a}	0.611	7.05 ^{a}
Goblet Cells number	Duodenum	35	22.92 ^{c}	25.17 ^{b}	30.62 ^{ab}	34.36 ^{a}	1.75	28.27 ^{a}
		42	25.11 ^{c}	27.35 ^{b}	30.09 ^{b}	35.70 ^{a}	1.65	29.56 ^{a}
	Jejunum	35	26.73 ^{c}	28.55 ^{bc}	33.81 ^{b}	38.51 ^{a}	2.05	31.90 ^{b}
		42	27.00 ^{c}	32.25 ^{b}	38.10 ^{ab}	40.20 ^{a}	2.33	34.39 ^{a}
	Ileum	35	29.32 ^{c}	36.17 ^{b}	38.33 ^{b}	45.05 ^{a}	1.94	37.22 ^{a}
		42	30.92 ^{c}	36.28 ^{b}	41.15 ^{b}	47.10 ^{a}	2.57	36.85 ^{a}

T0= Control, T1=0.2% ginger powder, T2=0.4% ginger powder , T3= 0.6% ginger powder. $^{a-c}$ Means within rows with different superscripts differ significantly (P≤ 0.05).

DISCUSSION

The minute level of ginger used in this study at level (0.2, 0.4, 0.6)% promoted body weight, body weight gain and feed conversion ratio at the treatments of ginger powder addition to diet compeer to the control group without ginger, and the best result occur in T3 compare to T2 and T1. While, the feed intake is lowest at T3, T2 and T1 respectively compare to the control group. This finding is in agreement with previous study [4] in which the level of ginger diet improve body performance and weight gain. In addition, the result of the current study is in agreement with [16], in which the addition of red ginger at level 1.5 % in the ratio give the highest broiler body weight and considerably lowest feed conversion ratio has been found that the red ginger

contain active compounds such as asiri oil and oleoresin all the compounds adding phytobiotic of ginger causing an improve in feed digestion.

It has been found that birds feed with supplementation of powder ginger revealed the best result of viability and broiler production index in T3, then T2, and T1 respectively. It has also been found that the lowest mortality rate in groups have be ginger powder in diet when compare to the control group suggesting that Ginger contains several compounds such as gingerol, gingerdiol, and gingerdione that possess strong antioxidant activity [9].

The lowest ratio of H/L revealed in the T3, T2 and T1 respectively compare to the control group which suggest that ginger good anti stress factor when added to diet. This finding is an agreement with the previous study [2], in which there was no significant effects on most of the WBCs parameters, except for WBCs, heterophile and heterophile/lymphocytes ratio. WBCs were significantly lower for groups given 0.5% ginger continuously than the other groups suggesting an improvement in cellular immunity of chickens supplemented with 0.5% ginger continuously. While this finding is in agreement with Tavakol [17] who revealed significant difference among the experimental groups in the number of white blood cells. The number of WBCs in the blood plasma showed a significant positive Ginger due to the stimulatory effect on the immune system through the intestinal absorption and share all types of WBCs.

The study revealed higher significantly improved in antibody titer against Newcastle Disease (ND), Infectious Bursal Disease (IBD), and Infectious bronchitis (IBV) in all groups that have addition ginger powder in feed compare with the control (T0) at all ages were test by ELISA, suggesting Ginger powder can improve the immune response to these vaccines. In contrast, [18] reported that adding ginger powder did not affect the titer of antibody against Newcastle disease. In addition, the effect of ginger on antibody response to NDV is similar to those reported by [19]. This finding is in agreement with Attia [2]. It has been found that 0.5% ginger supplementation intermittently increased antibody titer to NDV compare to the other groups, but contrast for IBD 0.5% continuous ginger supplementation decreased antibody titer to IBD compare to the other groups. The present study also revealed that the delayed type of hypersensitivity (DTH) for Newcastle disease (ND) was significantly increase in all the groups of ginger addition at 24 and 48 h of injecting compare with the control.

The results showed of small intestine length to body weight were significantly higher in the treatment T3 at age 35, and in T2 and T3 at age 42. In addition, the results of goblet cells number

were significantly increased in all the treatments of ginger addition of broiler duodenum at 35 and 42 days, thus the results recorded more number of goblet cells in the treatments T2 and T3 at age 35 and all the treatments at age 42 of jejunum. Ileum goblet cells also revealed high number in all the treatments of ginger addition at ages 35 and 42 days compare with the control. This represents an increase in the absorptive surface area of the intestine and thus an increased absorptive capacity resulting higher body weight gain and lower FCR in ginger supplemented group. This finding is in agreement with [20], in which the statistical analysis of the morphometric of the jejunum showed that values of mean villi length and width were significantly higher in ginger group. In addition the goblet cells mucin is a glycoproteins play role as defensive barrier of the intestinal mucus layer, and in the regular function of the epithelium of the gastrointestinal tract. So its development is influenced by the time of access to feed [21].

CONCLUSION

This study concluded that the ginger powder improve the body performance of broiler chickens, increase the body weight and feed conversion ratio. In addition ginger increase the availability and decrease the mortality rate and broiler production index. Ginger powder positive effect on the immune system by decrease the ratio of H/L and increase the antibody titers against the Newcastle Disease, Infectious Bursa disease and Infectious Bronchitis. Furthermore, the highest level of delayed hypersensitivity for Newcastle Disease was record in groups which supplementation the ginger powder to diet. The current study revealed that the T3 (0.6%) and T2 (0.4%) increase the goblet cell numbers in duodenum, jejunum, and ileum of small intestine. In addition in the most traits of the current study results the age 35 better than 42 and more economic therefore preferred rearing broilers till this age nor 42.

تأثير إضافة مسحوق الزنجبيل في عليقة دجاج فروج اللحم على تطور الحالة المناعية ومورفولوجيا الأمعاء الدقيقة

نوال كمال شكري محبوبة عبدالغني مصطفى [1] نضال عبدالغني مصطفى

قسم الثروة الحيوانية/ كلية الزراعة/ جامعة صلاح الدين-أربيل/ العراق

الخلاصة

أُجريت الدراسة باستخدام 300 طير فروج اللحم سلالة Ross-308 بعمر يوم واحد لتقييم تأثير إضافة مسحوق جذور الزنجبيل المجفف (Zingiber officinale) بنسب (0.2 و 0.4 و 0.6)٪ في المعاملات T1 و T2 و T3 على التوالي على الأداء الإنتاجي، والحالة المناعية ومورفولوجيا الأمعاء الدقيقة لدجاج فروج اللحم بأعمار مختلفة مقارنة مع مجموعة السيطرة (T0)دون إضافة مسحوق الزنجبيل.

أن وزن الجسم، الزيادة الوزنية للجسم، الحيوية، الدليل الإنتاجي والربح الاقتصادي أزداد معنويا (P≤0.05) بإضافة مسحوق الزنجبيل بشكل ملحوظ في T3 بأعمار (1-21) و (36-42) يوما. وكذلك تحسن معدل التحويل الغذائي (FCR) . بإضافة إلى ذلك أرتفع تشريح الأمعاء الدقيقة للخلايا الكأسية في (الاثني عشر، الصائم واللفائفي)، فرط الحساسية DTH و المعيار الحجمي للأضداد بالإيلايزا ضد حمى نيوكاسل (ND) ، جامبورو (IBD) وأمراض التهاب القصبات المعدية (IB) في دجاج فروج اللحم معنويا (P≤0.05) عند عمر 21 و 35 و 42 يوما. في حين انخفضت نسبة الهلاكات ونسبة الخلايا المتغايرة الى خلايا اللمفية (H/L) بشكل ملحوظ في معاملات إضافة مسحوق الزنجبيل خاصة في T3 مقارنة مع مجموعة السيطرة دون إضافة الزنجبيل.

REFERENCES

1. **Attia, Y.A. and Al-Harthi M.A., 2015**. Nigella seed oil as an alternative to antibiotic growth promoters for broiler Chickens. Europ. *Poult. Sci*., 79 (10):1399.
2. **Attia,Y.A., Hassan S.S., Abd-El-Hamid A.E. and Radwan M.W., 2017**. Response of broiler chickens to dietary supplementation of Ginger (*zingiber officinale*) continuously or intermediately in comparison with prebiotics. Egypt. *Poult. Sci.* Vol 37, (II): 523-543 .
3. **Azhir, D., Zakeri A. and Kargare-Rezapour A., 2012**. Effect of ginger powder rhizome on homural immunity of broiler chickens. Pelagia Research Library. European J. of Experimental Biology, 2 (6): 2090-2092.
4. **Barazesh, H., Pour M.B., Salari S. and Abadi T.M., 2013**. The effect of ginger powder on performance, carcass characteristics and blood parameters of broilers. International journal of Advanced Biological and Biomedical Research 1, (12): 1645-1651.

5. **Mohamed, A.B., Al-Rubaee M.A. and Jalil A.Q., 2012**. Effect of Ginger (*Zingiber officinale*) on Performance and Blood Serum Parameters of Broiler. International Journal of Poultry Science 11 (2): 143-146.

6. **Adeyemo, G.O., Olowookere I.J and Longe O.G., 2016.** Effect of Dietary Inclusion of Ginger (Zingiber officinale) Dried with Different Methods on Performance and Gut Microbial Population of Broiler Chicks. American Journal of Experimental Agriculture 11(4): 1-7.

7. **Ebrahimnezhad, Y., Azarakhsh V. and Salmanzadeh M., 2014.** The effects of ginger root (zingiber officiale) processed to different levels on growth performance, carcass characteristics and blood biochemistry parameters in broiler chickens. Bull. Env. Pharmacol. Life Sci., Vol 3 (5): 203-208.

8. **Dieumou, E.F., Teguia A., Kuiate J.R, Tamokou J.D., Fonge N.B. and Dongmo M.C., 2009**. Effects of ginger (Zingiber officinale) and garlic (Allium sativum) essential oils on growth performance and gut microbial population of broiler chickens. Livestock Research for Rural Development 21 (8).

9. **Zhang, G.F., Yang., Z.B., Wang, Y., Yang, W.R., Jiang, S.Z. and Gai, G.S., 2009.** Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poult. Sci.,* 88, (10): 2159-2166.

10. **AL-Murrani, W.K., Hamed, H.; AbdulGani G. and Omran A.H., 1995**. Some aspects of genetic resistance to S. typhimurium in native and white leghorn chickens. Dirasat, vol. 22. Jordon.

11. **Cook, F. W., 1959.** Staining fixed preparation of chicken blood cells with combination May-Grünwald-Right-Phloxine B stain. Avian Dis. 3:272–290.

12. **Uni, Z.; Noy Y. and Sklan D., 1999.** Post hatch development of small intestinal function in the Poultry. *Poult. Sci*., 78: 215-221.

13. **Tako, E.; Ferket P.R. and Uni Z., 2004**. Effects of inovo feeding of carbohydrates and beta-hydroxy beta-methylbutyrate on the development of chicken intestine. *Poult. Sci*.; 83 (12): 2023-2028.

14. **SAS, Statistical analysis system. 2005**. User's guide for personal computer. Release 8.2 SAS Institute Inc. Cary, NC, USA.

15. Duncan, D. B., 1955. Multiple range test and multiple F tests. Biometrics 11, 1-42.

16. Herawati. 2010. The effect of feeding red ginger as phytobiotic on body weight gain, feed conversion and internal organs condition of broiler International Journal of Poult. Sci. 9 (10): 963-967.

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

18. Farhumand, P. and Fazli B., 2009. Comparison effect of ginger powder and nettle powder by antibiotic growth promoter on immunity parameters in broiler. *Poult. Sci*. (87): 2629-2635.

19. Khan, R.U., Naz S., Nikousefat Z., Tufarelli V., Javdani M., Qureshi M. S. and Laudadio V., 2012. Potential applications of ginger (*Zingiber officinale*) in poultry diets. *World's Poult. Sci.* J., 68: 245-252.

20. Karangiya, V.K., Savsani H.H., Patil1 S.S., Garg D.D., Murthy K.S., Ribadiya N.K. and Vekariya S.J., 2016. Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. Veterinary World, 9 (3): 245-250.

21. Uni, Z., A. Smirnov, and D. Sklan. 2003. Pre- and Posthatch Development of Goblet Cells in the Broiler Small Intestine: Effect of Delayed Access to Feed. *Poultry Science,* 82:320–327.