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The effect of adding different concentrations of fresh onion juice to the drinking water of broiler chickens on productive and immune performance

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Abstract

Poultry scientists are currently searching for feed additives capable of combating pathogens, enhancing bird immunity, improving growth, and maintaining product quality. This study aims to evaluate the effect of different concentrations of fresh onion juice on growth performance and immune response parameters in broiler chickens. 170 one-day-old, unsexed Ross 308 broiler chicks were obtained from a hatchery in Babil province, Iraq. On the first day of the experiment, 20 chicks were sacrificed for blood collection to measure maternal immunity against Newcastle disease virus (NDV) by indirect ELISA. The remaining chicks were distributed into three equal treatment groups, each with five replicates, with 10 chicks per pen in a completely randomized design. All chicks in all experimental groups were fed standard balanced diets. According to the age stage of the birds, fresh onion juice was added to the drinking water of chicks in experimental groups 1, 2 and 3 at concentrations of 0, 1.5% and 2.5% of water, respectively. All chicks were vaccinated against Newcastle disease (ND) using ND vaccine three times on days 7, 17 and 27, respectively, by intraocular route. Feed consumption and body weight were recorded weekly and used to calculate feed conversion ratio (FCR). At the end of the experiment (after 35 days), blood was collected from the chicks in each replicate of each group for the purpose of evaluating humoral immunity by indirect ELISA, cellular immunity by counting CD8 by competitive ELISA, and stress rate by measuring the heterophil/lymphocyte ratio. Then, four chickens from each pen were randomly selected for slaughter to evaluate the lymphoid organs weight index. An increase in the means weekly body weight, weekly feed consumption and weekly feed conversion ratio were observed ($p < 0.05$) at 1.5% and 2.5% of fresh onion juice in broiler drinking water. Also, there is a significant difference at ($p < 0.05$) in the average antibody titer, CD8 concentration, relative weight of lymphoid organs and heterophil/lymphocyte ratio between the two groups treated with onion juice at a rate of 2.5% and 1.5% respectively and control group. Based on these results, the study concluded that the addition of fresh onion juice in broiler drinking water has positive effects in improve the growth performance, immunity and decrease stress of broiler chicken. Also, there isn't effect of high concentration of fresh onion juice in study parameters except in antibody titer. For that this study, recommended to addition of 1.5% fresh onion juice in broiler drinking water as safe natural promoter and antistress. While mean addition 2.5% of it as inducer of humoral immunity.

Keywords: Onion, broiler chickens, immunity, productive, performance

Introduction

Poultry farming is widespread throughout the world to meet the increasing demand for animal protein due to the increasing population. However, there are several factors that limit the expansion and progress of poultry farming in some countries, including the level of nutrition, control of environmental factors within poultry houses, as well as biosecurity measures that must be applied to control diseases (Korver, 2023) [16]. However, disease control remains the major threat facing the poultry industry, as pathogenic microorganisms are ubiquitous in feed, water and the environment, making them difficult to control (Aruwa and Sabiu, 2024) [6]. When these pathogens enter the digestive tract, they disrupt the digestive system's functions, including motility, digestion, and absorption of nutrients (Cross *et al.*, 2007) [7], this leads to a deterioration in the health of the chicks and reduces their growth and productivity

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(Sundström *et al.*, 2014) ^[28]. Antibiotics used as growth promoters in poultry feed, but, that lead to controversies, such as drug resistance and residues in the meat (Adeyemi *et al.*, 2021) ^[1]. Therefore, researchers are currently seeking safety feed additives capable of combating pathogens, enhancing growth performance and maintaining product quality. Natural feed additives, particularly phytobiotics which possess antioxidant and antimicrobial properties used as growth promoter and boosting immunity (Farahani *et al.*, 2015; Mulugeta *et al.*, 2019; Malematja *et al.*, 2022; Phillips *et al.*, 2023) ^[12, 20, 18, 23]. Many studies have been conducted to investigate the possibility of onion extracts improving the health and productivity of chickens, and the results of these studies were positive in improving poultry production in terms of body weight gain and feed conversion (Demir *et al.*, 2003; Ahmad *et al.*, 2008; Malematja *et al.*, 2022; Malematja *et al.*, 2023) ^[8, 3, 18, 19]. Due to the presence of many organic compounds, flavonoids and phenolic acids in onions with antibacterial, antioxidant and lipid-lowering properties, onion is considered a good growth promoter in poultry (Srinivasan *et al.*, 2004; Joe *et al.*, 2009) ^[27, 15]. Despite, further research on onions as a food additive, there is still a clear knowledge gap regarding the direct effects of fresh onion juice supplied via drinking water on broiler chicken growth and immunity, this study aimed to determine the optimal concentration of fresh onion juice added to broiler drinking water to improve production and immune performance.

Materials and Methods

The experiment was conducted at the College of Veterinary Medicine, Al-Qasim Green University, Department of Pathology and Poultry Diseases, in Babil province, central Iraq. The protocol of animal handling was approved by the Al-Qasim Green University Animal Research Ethics Committee (No 2650 on 21/10/2024).

The experiment extended from between March and April 2025, one hundred seventy, one day old broiler chicks unsexed (Ross308) with average weight was 45 g purchased from one hatchery in Babil Province to carry out this experiment. At day one, twenty chicks bled for blood samples collection for estimation the maternal derived antibody titer (MDAT) against NDV, by using indirect Enzyme Linked Immunosorbent Assay (ELISA). The rest one hundred fifty chicks were randomly distributed into three equal groups, fifty chicks for each group, each group include five replicates (replicate=10 chicks). Chicks in each group were reared in sterile separated floor pens (1×5×1m³) under good hygienic condition for 35 days. The experiment pens were supplied by all breeding requirements according to routine management practice as out lined by the National Research Council requirements (NRC, 1994). All chicks in all experimental groups were fed ad libitum with broiler standard balanced diets, according to the age stage of the birds according to the (NRC, 1994). Fresh onion juice has been added to the drinking water of chicks in experimental groups 1, 2 and 3 at concentrations of 0, 15 and 25 ml/liter of water, respectively. All chicks were vaccinated against Newcastle disease (ND) using ND vaccine (Poulshot NDV LaSota (1×106.0 EID50 Cavac/ Korea) three times on days 7, 17 and 27, respectively, by intraocular route. (Ahmadi *et al.*, 2007) ^[4]. Fresh onion bulbs (*Allium cepa*) were purchased from local markets in Babil Governorate, Iraq. They were then cleaned, peeled, grated into small pieces, and thoroughly squeezed using a fruit juicer machine. Onion juice is prepared daily before serving it to the chicks, according to the concentration

prescribed for each group. Using a digital balance, live body weight (LBW) was measured in g/chick for all birds in each treatment group immediately after hatching and then at weekly intervals for five consecutive weeks of the experimental period. The mean BWG (g/bird) was calculated at the end of each week and expressed as BWG=BWf-BWi; where BWf represents the body weight at the end of the week, and BWi indicates the body weight at the beginning of the week. Additionally, during rearing, the feed intake (FI; g/bird) was weekly recorded as the difference between feed supplied and the remaining feed in each feeder, and the total FI was calculated at the end of the experiment. The FCR was calculated at the end of the investigation as the amount of feed consumed per unit of body gain (g feed/g gain).

At the end of the experiment (on day 35), two chickens were randomly selected from each replicate to collect 4 ml of blood from the wing vein using (5) ml sterile disposable syringes. This sample was divided into two parts, one of which was placed in a tube without anticoagulant to separate the serum for measuring the humoral immunity parameter by indirect ELISA (ID Screen® Newcastle disease Indirect ELISA Kit ID. Vet/ France), and cellular immunity by counting CD8 by competitive ELISA (Chicken cluster of differentiation (CD8) ELISA kits (competitive ELISA kit) BT LAB/ China). The other part of the sample was placed in a tube containing an anticoagulant to measure the heterophil/lymphocyte ratio as stress indicator have been measured by automated veterinary hematology analyzer BC-5000 vet five part blood test medical CBC machine according to (Post *et al.*, 2003) ^[24]. Four chickens from each pen were then randomly selected for slaughter to assess the lymphocyte weight/body weight index, which was calculated using the formula: organ weight in grams × 1000 / final body weight in grams (Sharma *et al.*, 1989) ^[26].

The Statistical Analysis

Data were analyzed with a one-way analysis of variance (ANOVA) using the GLM procedure in SPSS (SPSS Inc., Chicago, IL, USA) after Shapiro-Wilk's test was used to verify the normality and Levene's test was used to verify homogeneity of variance components between experimental treatments. The replicate, or the individual bird, served as an experimental unit for all statistical analyses. The significant difference between the mean values of body weight, body weight, feed intake, feed conversion ratio, mean of immunity were tested using Duncan's multiple range test (Duncan, 1955), and the variation in the data was expressed as pooled SEM, and the significance level was set at $p < 0.05$.

Results

Results of the effect of adding different concentrations of fresh onion juice to the drinking water of broiler chickens on means weekly body weight of the chicks throughout the experimental period extending from the first week to the fifth week of the chicks' age are presented in Table1. The present study showed that adding fresh onion juice to the drinking water of broiler at a rate of 1.5% and 2.5% respectively there was a significant difference at the $p \leq 0.05$ in the means weekly body weight in compared with control group.

While the results did not show any significant difference in the weekly weight gain rate of the chicks throughout the experiment period between the two experimental groups treated with adding onion juice to the drinking water at a rate of 1.5% and 2.5% respectively and the control group except

for the first and third week where a significant difference was recorded at the $p \leq 0.05$ as shown in Table 2.

Table 1: Effect of onion juice added to drinking water at (0, 1.5 and 2.5%) on average of broiler body weight (gram/bird/week)

Age/week	Body weight (g)±str. error when onion juice added to drinking water		
	0%	1.5%	2.5%
1 st	167.4±0.791 ^{b*}	170.6±0.581 ^a	171.8±0.46 ^a
2 nd	394.8±2.53 ^b	404.4±2.24 ^a	404.2±2.56 ^a
3 rd	815.3±1.60 ^b	848.8±2.53 ^a	847±1.54 ^a
4 th	1347.8±3.85 ^b	1389.4±7.02 ^a	1392.7±5.21 ^a
5 th	1963.7±1.46 ^b	2007.8±5.57 ^a	2014.5±3.83 ^a

*The difference small letters horizontally referred to significant difference $p \leq 0.05$

Table 2: Effect of onion juice added to drinking water at (0, 1.5 and 2.5%) on average of weight gain (gram/bird/week)

Age/week	Weight gain (g)±str. error when onion juice added to drinking water (%)		
	0%	1.5%	2.5%
1 st	127.4±1.146 ^{b*}	130.2±0.592 ^a	132.3±0.42 ^a
2 nd	227.4±2.93 ^a	233.8±2.096 ^a	232.4±2.63 ^a
3 rd	420.5±2.522 ^b	440.0±2.71 ^a	442.8±3.60 ^a
4 th	533.5±7.51 ^a	546.0±4.79 ^a	545.3±4.57 ^a
5 th	614.9±7.01 ^a	618.4±6.32 ^a	622.2±7.08 ^a

*The difference small letters horizontally referred to significant difference $p \leq 0.05$

The results showed that the highest rate of feed consumption per week was for the control group chicks, where it recorded a rate of 139.8±0.512 compared to the two groups treated with adding onion juice to the drinking water at a rate of 1.5% and 2.5%, which recorded a rate of 135.2±0.679 and 135.1±0.622 respectively. The feed consumption mean of chicks in the second week was almost equal among the three experimental groups. While there was a significant difference in the feed consumption mean at the level of $p \leq 0.05$ among the experimental groups during the third, fourth and fifth weeks, as shown in Table 3.

Table 3: Effect of onion juice added to drinking water at (0, 1.5 and 2.5%) on average of feed consumption (gram/bird/week)

Age/week	Feed consumption (g)±str. error when onion juice added to drinking water		
	0%	1.5%	2.5%
1 st	139.8±0.512 ^a	135.2±0.67 ^b	135.1±0.62 ^b
2 nd	321.5±1.565 ^a	317.4±1.146 ^a	317.5±1.92 ^a
3 rd	698.8±5.73 ^a	668.8±11.95 ^b	658.0±7.72 ^b
4 th	1006.0±3.78 ^a	981.8±4.66 ^b	967.3±2.72 ^c
5 th	1195.8±4.55 ^a	1158.8±2.28 ^b	1148.0±2.00 ^c

*The difference small letters horizontally referred to significant difference $p \leq 0.05$

Table 4: Effect of onion juice added to drinking water at (0, 1.5 and 2.5%) on average of feed conversion

Age/week	Feed conversion±str. error when onion juice added to drinking water		
	0	1.5	2.5
1 st	1.09±0.0103 ^a	1.03±0.0061 ^b	1.02±0.0070 ^b
2 nd	1.41±0.022 ^a	1.36±0.015 ^a	1.36±0.018 ^a
3 rd	1.66±0.020 ^a	1.52±0.025 ^b	1.48±0.019 ^b
4 th	1.88±0.029 ^a	1.79±0.016 ^b	1.77±0.013 ^b
5 th	1.94±0.026 ^a	1.87±0.017 ^b	1.84±0.020 ^b
Sample no	10		

*The difference small letters horizontally referred to significant difference $p \leq 0.05$

Also, the results of current study showed that there is decrease a significant difference at the $p \leq 0.05$ in the average weekly Feed conversion ratio between treatment groups (G2 and G3) and control group (G1) along experimental period except in 2nd week, as shown in Table 4.

From Table 5, we note that there is a significant difference at $p \leq 0.05$ in the humoral immunity in favor of the group of chicks treated with the addition of onion juice at a rate of 2.5% to the drinking water at the expense of the chicks in the two groups treated with onion juice at a rate of 1.5% and 0%, respectively. On the other the hand, we note a significant difference of 0.05% in the average CD8 concentration of the two groups treated with onion juice at a rate of 2.5 and 1.5% compared to the group treated with a rate of 0%. Meanwhile, we observe a significant difference in the averages of Bursa index, Thymus index, Spleen index and Hetrophil/lymphocyte between the two groups treated with onion juice at a rate of 2.5 and 1.5% and the control group treated with 0% onion juice.

Table 5: Effect of onion juice added to drinking water at (0, 1.5 and 2.5%) on average of immunity parameters and Hetrophil/lymphocyte ratio

Parameter	Concentration of onion juice added to drinking water		
	0	1.5	2.5
Humoral immunity	4476.7±237.6 ^b	4797.2±234.9 ^b	5340.3±188.9 ^a
CD8 concentration	2290.8±204.7 ^b	3058.2±212.4 ^a	3665.4±201.4 ^a
Bursa index	0.22±0.011 ^b	0.27±0.011 ^a	0.26±0.004 ^a
Thymus index	0.27±0.086 ^b	0.32±0.0083 ^a	0.34±0.0112 ^a
Spleen index	0.15±0.004 ^b	0.17±0.005 ^a	0.18±0.005 ^a
Hetrophil/lymphocyte	0.64±0.040 ^a	0.52±0.027 ^b	0.56±0.021 ^{ab}

*The difference small letters horizontally referred to significant difference $p \leq 0.05$

Discussion

The results of this study showed that there was a significant effect at the ($p \leq 0.05$) of onion juice when added to the drinking water of broiler chickens at concentrations of 2.5% and 1.5% respectively, especially on the average weekly body weight, the average weekly feed consumption rate, and the average feed conversion ratio when compared to the control group. This positive effect of fresh onion juice may be attributed to its content of organic compounds, flavonoids, and phenolic acids with proven effectiveness as antibacterial, antioxidant, and lipid-lowering agents. Which is known for its role in promoting poultry growth performance by enhancing intestinal health and its efficiency, through improving the ecological balance of the intestines by increasing the normal micro flora and reducing harmful microorganisms. On the other hand, it enhances the effectiveness of enzymes such as catalase, glutathione peroxidase, and superoxide dismutase, which are known for their role in reducing oxidative stress in poultry tissues, leading to improved bird health and increased productivity. This justification is consistent with many studies (Omar *et al.*, 2020; Enoka *et al.*, 2020; Adeyemi *et al.*, 2021; Sigolo *et al.*, 2021; Kousar *et al.*, 2024) [22, 11, 1, 25, 17]. The results of the current study are consistent with the results of several previous studies, most notably (Goodarzi *et al.*, 2013; An *et al.*, 2014; Farahani *et al.*, 2015; Aditya *et al.*, 2016; Omar *et al.*, 2020 [13, 5, 12, 2, 22] (who confirmed that adding onion extracts to poultry water or feed has a positive effect on their productive and health performance. However, the results of this study contradicted the results of other studies that indicated no significant effects of onion extracts on the

productive performance of broiler chickens). Dublado *et al.*, 2016; Malematja *et al.*, 2023) [9, 18].

In our study, immunity was improved with higher antibody titer, CD8 concentration and relative weight of lymphoid organs in groups that supplemented with 1.5% and 2.5% fresh onion juice supplemented as compared to control group during serological analyses which indicate significant results in the immunological response of broiler chickens vaccinated against the Newcastle virus. That may be attributed to the bioactive compounds in onion, such as flavonoids and sulfur-containing molecules, which are known to modulate growth performance and immune function in broilers by increasing weight of lymphoid organs, the oversized follicles promoting the proliferation macrophages, lymphocytes and of plasma cells lead to an increased immune globulin synthesis. This leads to improved antibody production (humoral immunity) and a stronger cellular response to pathogens (Hanieh *et al.*, 2010; Malematja *et al.*, 2022) [14, 18]. The results of present study, were consistent with Hanieh *et al.* (2010) [14] and Omar *et al.*, (2020) [22] who reported that humoral immune function was improved by supplementing onions in the chicken diet after vaccinating the birds with the Newcastle Virus (NDV). On the other hand, there is a significant difference in heterophil/lymphocyte ratio between the group which supplemented with 1.5% of onion juice and control groups, that may be attributed to onion juice rich in antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase, which are known for their role in reducing oxidative stress in poultry tissues (Omar *et al.*, 2020; Enoka *et al.*, 2020; Adeyemi *et al.*, 2021; Sigolo *et al.*, 2021; Kousar *et al.*, 2024) [22, 11, 1, 25, 17].

Conclusions

Based on these results, the study concluded that the addition of fresh onion juice in broiler drinking water has positive effects in improve the growth performance, immunity and decrease stress of broiler chicken. Also, there isn't effect of high concentration of fresh onion juice in study parameters except in antibody titer. For that this study, recommended to addition of 1.5% fresh onion juice in broiler drinking water as safe natural promoter and antistress. While mean addition 2.5% of it as inducer of humoral immunity.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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