

## The Effect of Artemisia on Immune Response and Productive Performance Against Newcastle Disease in Broiler Chickens

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### ABSTRACT

Prevention of Newcastle disease has received a lot of interest across the world. The high productivity performance of the commercial chickens' breeds has negative effects on the immune system and animal welfare. As a result, the current study aimed to investigate the benefits of adding Artemisia powder at levels of 0.5% and 1% to broilers' feed as a growth and health promoter. A total of 120 commercial broiler chickens were grown on the floor in a chicken house and separated into three groups, including one control and two treatment groups. Each group contained 40 chickens subdivided into two replicates. The three groups, namely G1 (chickens without Artemisia powder, as a control group), G2, and G3 in which chickens were fed with basal diet plus 0.5% and 1% Artemisia powder, respectively, were differentiated based on their diet throughout 35 days of the experiment. The measured parameters included the immune response to Newcastle disease vaccine, blood biochemical parameters, and growth performance as well as relative weight for the spleen and bursa of Fabricius. A diet containing 1% Artemisia powder significantly improved antibody titer against Newcastle disease, body weight, and weight gain. Thus, the addition of 1% of Artemisia powder to the broiler's diet can improve immune response against Newcastle disease and growth performance.

**Keywords:** Artemisia, Broiler chicken, Newcastle disease, Productive traits

### INTRODUCTION

Considering the poultry diet, feed additives, including antibiotics, enzymes, diet dyes, flavors additives, probiotics, (Vahdatpour and Babazadeh, 2016), phytobiotics (Saeed et al., 2018), and antioxidants (Pirgozliev et al., 2019) are added to enhance poultry production performance although they are not nutritious. These substances are added to a diet mainly for the improvement of feed intake and growth rate in chickens (Bali et al., 2011; Abouelfetouh and Moussa, 2012; Ahsan et al., 2018). Most growth promoter antibiotics were banned by the European Union countries in 2006 due to their remains in body tissues and the development of pathogen resistance (Millet and Maertens, 2011). Researchers have investigated the use of aromatic plants as an alternative to antibiotics. Aromatic plants or herbs as feed additives are used widely in the Middle East for a

long time ago helping to enhance food taste (Saeed et al., 2017; Daramola, 2019; Christaki et al., 2020). Medical plants supplementation in the broiler chicken diet could affect performance characteristics, serum metabolites, and antioxidant status (Kostadinović et al., 2015; Jin et al., 2020). According to the World Health Organization, about 80% of medical plants in humans are used for disease prevention and treatment particularly in developing countries (Deniz et al., 2021).

Chicken production is mainly correlated to gastrointestinal tract (GIT) efficiency, where its main role is feed digestion and metabolism (Ravindran and Abdollahi, 2021). Thus, GIT health is playing a substantial role in productivity as it affects the utilization of energy, proteins, and consequently disease resistance. Phytogetic feed additives (PFAs) are substances of plant origin used as diet additives in the poultry industry for the improvement of performance characteristics (Noonan,

2018). In modern animal production, PFAs are used for their antimicrobial effect, digestibility enhancement, and performance improvement (Hafeez et al., 2016; Ahsan et al., 2018). Artemisia is a class of plant that belongs to the Asteraceae family and is used as human and animal medicine. The nutrition contents of Artemisia are presented by Iqbal et al. (2012) which includes carbohydrates, fibers, fat, protein, phytate, tannin, and tocopherol. Artemisia has been utilized in poultry production as an enhancer of weight gain, feed conversion ratio (Gholamrezaie et al., 2013), and gut microbiota (Saracila et al., 2018; Song et al., 2018; Panaite et al., 2019). Abdullah and Al-Barwary (2020) indicated that the addition of Artemisia to the broilers' diet can lead to improved performance, gut morphology, and serum biochemical parameters during a coccidiosis challenge. Saracila et al. (2018) found that intestinal microflora improved in broilers reared under heat stress after the administration of Artemisia in the diet. Guo et al. (2020) found that Artemisia could improve growth performance and antioxidant function in broilers. Liu et al. (2009) demonstrated that Artemisia inhibited the proliferation of Newcastle disease virus in chicken embryos in an *in vivo* study. In Iraq, the commercial poultry industry aims to reach the highest body weight gain from feed intake. However, productivity and immune response for diseases are adversely correlated (Lwelamira et al., 2009; Sahib, 2021).

Many countries have tended to minimize or prohibit the chemical components because of their deleterious side effects on both animals and humans (Hao et al., 2014; Osman et al., 2018). Therefore, the aim of the current research was to investigate the effects of adding Artemisia powder at levels of 0.5% and 1% to the basal diet on immune response against Newcastle disease and the productive performance of broiler chickens.

## MATERIALS AND METHODS

### Ethical approval

All the procedures involved in the current study were confirmed by the scientific and animal care committee at the University of Kufa, Iraq (Certificate number: 100821.139).

### Chickens

This experiment was conducted at the poultry farm of the University of Kufa, Iraq. A total of 120 Cobb 500 broiler chickens aged one day old were purchased from a local hatchery in Najaf, Iraq. One-day-old chickens with

an average weight of 39.8 g were randomly split into three nutritional groups with two replicates (20 birds per replicate). All the management requirements (temperature, light, and ventilation) were met based on the Cobb 500 production guide management (2021). Chicks were raised on wood shaving floor pens. The first group (G1) was provided with a basal diet as a control group. While the second group (G2) and the third group (G3) were daily fed on a basal diet plus 0.5% and 1% Artemisia powder respectively. Feed and water were available *ad libitum*. Two formulas of diets (starter and finisher) were utilized during 35 days of the experiment (Table 1). All birds received a companioned Newcastle disease (ND) (B1 strain, Poulvac®, USA) and infectious bronchitis vaccine (Poulvac®, USA) by spraying each of them on the first day at the hatchery.

On the other hand, booster dose was administered orally in water, these vaccines included ND (Lasota strain Poulvac®, USA) on day 10 as well as Gumboro (IBD2, Bursine®, USA) on days 14 and 23 (Lasota strain of ND, Poulvac®, USA). Moreover, Vitamin C was administered (1 gm/liter) orally for 3 days after each vaccination. The chickens cared in accordance with animal welfare principles and with attention to ethics committee roles.

**Table 1.** Composition of diet in broiler chickens during 5 weeks of rearing

Ingredient (%)	Initial diet (1- 20 day)	Closer diet (21-35 day)
Corn (yellow)	53	44
Soybean meal (48% protein)	30	21
Wheat	10	26
Protein concentrate	5	5
Oil	1	3
Premix (VAPCO®, Jordan)	0.2	0.2
Limestone	0.5	0.5
Salt	0.2	0.2
Dicalcium phosphate	0.1	0.1
Overall	100	100
<b>Chemical analysis</b>		
Energy (kcal/kg)	2951.5	3128.5
Crude protein (%)	22	19

The balanced diet based on the NRC (1994). Premix is a mixture of vitamins and minerals.

### Production traits

The electronic balance was used to measure live body weight and feed consumption to calculate body weight gain and feed conversion ratio at weekly intervals (Aguilar *et al.*, 2013). However, the mortality rate was recorded daily as it occurred.

### Blood collection and analysis of cells count and enzymes

About 1 ml blood sample from brachial vein was collected from all chickens at age of 20 and 35 days by one-use syringe then transferred to test blank tubes to separate sera after clotting blood by centrifuge (10 minutes at 3000 rpm). In the next step, serum was frozen at  $-20^{\circ}\text{C}$  according to Mitchell and Johns (2008). Antibodies of Newcastle disease were measured in serum by utilizing a specific ELISA Kit (Proflok<sup>®</sup>, USA) run by following the instructions of the manufacturer. Blood components (WBCs and RBCs) measures were conducted using counter medionic cells. Blood biochemistry analyses of total protein, albumin, Hb, and AST were achieved by Micro Lab 300 analyzer (Merck<sup>®</sup>, France) and using kits from Merck (France). By following the instructions of the manufacturer after mixing the sample with provided reagents with the kits, and then the mixture was brought up to  $37^{\circ}\text{C}$  by incubation for 5 minutes. Measurement was achieved with equivalent wavelength to the designated parameters.

### Measurement of lymphatic organs

On day 35 of age, three chicks were randomly taken from each replicate and slaughtered after a cervical dislocation to avoid pain. The weight of each chick was measured using a digital balance. The internal organs, such as the spleen, bursa of Fabricius were weighted after collection by digital balance to calculate the ratio between them. Remove feather, head, legs, and viscera and kept only the carcass.

### Statistical analysis

The obtained data were analyzed in a complete randomized design (Steel and Torrie, 1980). These data were analyzed by one way ANOVA test following the procedure of the general linear model (SAS, system-version 9.1). Duncan's multiple range tests were employed to determine the differences among group means at a

significance level of  $p < 0.05$ . The following is the model:  $Y_{ij} = \mu + T_i + e_{ijk}$ , where,  $Y_{ij}$  represents individual observation,  $\mu$  is the overall mean,  $T_i$  indicates the effect of treatment ( $i = 1, 2, 3$ ), and  $e_{ijk}$  signifies a random error.

## RESULTS

The effects of Artemisia powder additive during the experimental period on immune response (antibodies titer) against Newcastle disease are shown in Figure 1. The antibody titers increased significantly in the chickens fed with Artemisia 1% at 35 days of age, compared to other groups ( $p < 0.05$ ). On the other hand, 0.5% of Artemisia did not lead to a significant increase regarding antibody titers ( $p < 0.05$ ).

The results of Artemisia's effects on the blood cells count and some biochemical tests are presented in Figure 2. There was a significant difference among treatment groups in terms of red blood cells (RBC) counts with the peak ( $3.2 \pm 0.04$ ) in the group fed 1% Artemisia ( $p < 0.05$ ). The Hb significantly increased in diet plus Artemisia with 0.5% and 1%, compared to the diet without Artemisia additive ( $p < 0.05$ ). White blood cells (WBC) count was significantly higher ( $32 \pm 0.7$ ) in the control group, compared to other groups (Artemisia 0.5% and 1%). Aspartate aminotransferase (AST) measurements revealed significant differences ( $p < 0.05$ ) and recorded a peak in the basal diet without Artemisia ( $242.5 \pm 2.02$ ), compared to chickens fed 0.5% Artemisia ( $218.5 \pm 4.47$ ). There were no significant differences among groups regarding both albumin and total blood protein.

The final body weight at 35 days of age enhanced significantly in G3 and G2 respectively, compared with G1 ( $p < 0.05$ , Figure 3). Weight gain of broiler chickens for all-treatments groups are presented in Table 2 and the total gain at the end of the experiment (day 35) is presented in Figure 4. The body weight gain was significantly different at week 5 when the highest was in G3 ( $764.9 \pm 32.9$  gm). The second highest record for body weight gain was for G2 ( $679.40 \pm 33.09$ ) and then the least was for G1 ( $556.20 \pm 12.85$ ). There were no significant differences among the three groups in terms of feed consumption and feed conversion (Tables 3 and 4). The same trend was true for the relative weight of lymphatic organs (bursa of Fabricius and spleen) among the three groups ( $p > 0.05$ , Table 5).

**Table 2.** Weight gain (g) of broiler chickens after dietary supplementation with different levels of *Artimasia* during 5 weeks of growing

Treatment	Age	First week	Second week	Third week	Fourth week	Fifth week	Total
G1 (control)		130.50±2.12 <sup>a</sup>	218.50±5.70 <sup>a</sup>	417.10±6.54 <sup>b</sup>	469.60±7.73 <sup>a</sup>	556.20±12.85 <sup>c</sup>	1791.90±8.30 <sup>b</sup>
G2 ( <i>Artemisia</i> 0.5%)		127.70±1.54 <sup>a</sup>	217.30±3.30 <sup>a</sup>	424.50±4.06 <sup>ab</sup>	469.40±6.51 <sup>a</sup>	679.40±33.09 <sup>b</sup>	1918.30±40.90 <sup>ab</sup>
G3 ( <i>Artemisia</i> 1%)		127.70±1.15 <sup>a</sup>	214.20±1.91 <sup>a</sup>	436.60±3.85 <sup>a</sup>	466.40±9.78 <sup>a</sup>	764.90±32.95 <sup>a</sup>	2009.80±40.60 <sup>a</sup>

Mean (gm) ± standard errors, Alteration in superscript letters in each column refers to a significant difference between treatments at  $p < 0.05$ .

**Table 3.** Feed intake (g) of broiler chickens after dietary supplementation with different levels of *Artimasia* during 5 weeks of growing

Treatment	Age	First week	Second week	Third week	Fourth week	Fifth week	Total
G1 (control)		157.50± 4.50 <sup>a</sup>	352± 9 <sup>a</sup>	666.90± 15 <sup>a</sup>	821± 20 <sup>a</sup>	1081± 25 <sup>a</sup>	3078.40± 73.50 <sup>a</sup>
G2 ( <i>Artemisia</i> 0.5%)		156.40± 2.50 <sup>a</sup>	371± 8 <sup>a</sup>	657.10± 13 <sup>a</sup>	813.50± 19.50 <sup>a</sup>	1082.50± 24.50 <sup>a</sup>	3080.50± 67.50 <sup>a</sup>
G3 ( <i>Artemisia</i> 1%)		157.40± 4.50 <sup>a</sup>	362± 9 <sup>a</sup>	673.70± 13 <sup>a</sup>	814± 18 <sup>a</sup>	1052.50± 24.50 <sup>a</sup>	3059.60± 69 <sup>a</sup>

Mean ± standard errors, Alteration in superscript letters in each column refers to a significant difference between treatments at  $p < 0.05$ .

**Table 4.** Feed conversion ratio of broiler chickens after dietary supplementation with different levels of *Artimasia* for 5 weeks

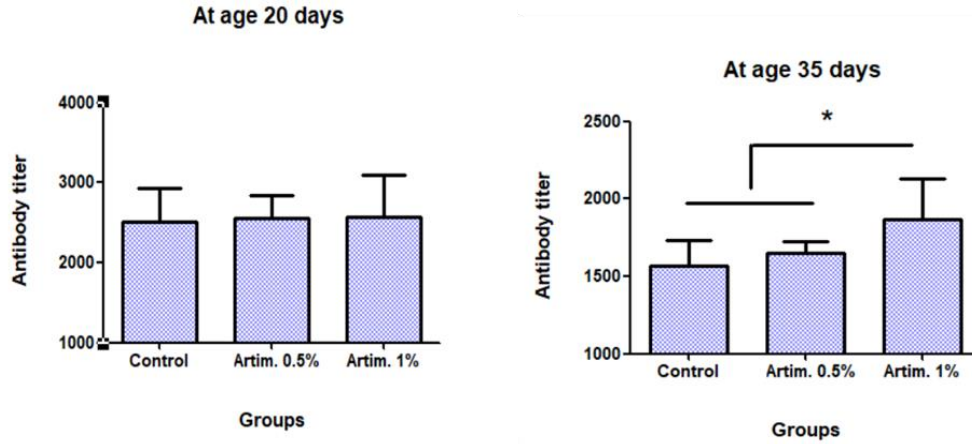
Treatment	Age	First week	Second week	Third week	Fourth week	Fifth week	Total
G1 (control)		1.206± 0.022 <sup>a</sup>	1.610± 0.021 <sup>a</sup>	1.598± 0.001 <sup>a</sup>	1.751± 0.100 <sup>a</sup>	1.944± 0.067 <sup>b</sup>	1.622± 0.042 <sup>a</sup>
G2 ( <i>Artemisia</i> 0.5%)		1.224± 0.016 <sup>a</sup>	1.707± 0.034 <sup>a</sup>	1.548± 0.057 <sup>a</sup>	1.733± 0.005 <sup>a</sup>	1.595± 0.045 <sup>a</sup>	1.561± 0.011 <sup>a</sup>
G3 ( <i>Artemisia</i> 1%)		1.233± 0.049 <sup>a</sup>	1.689± 0.024 <sup>a</sup>	1.543± 0.026 <sup>a</sup>	1.745± 0.048 <sup>a</sup>	1.378± 0.043 <sup>a</sup>	1.517± 0.021 <sup>a</sup>

Mean ± standard errors, Alteration in superscript letters in each column refers to a significant difference between treatments at  $p < 0.05$ .

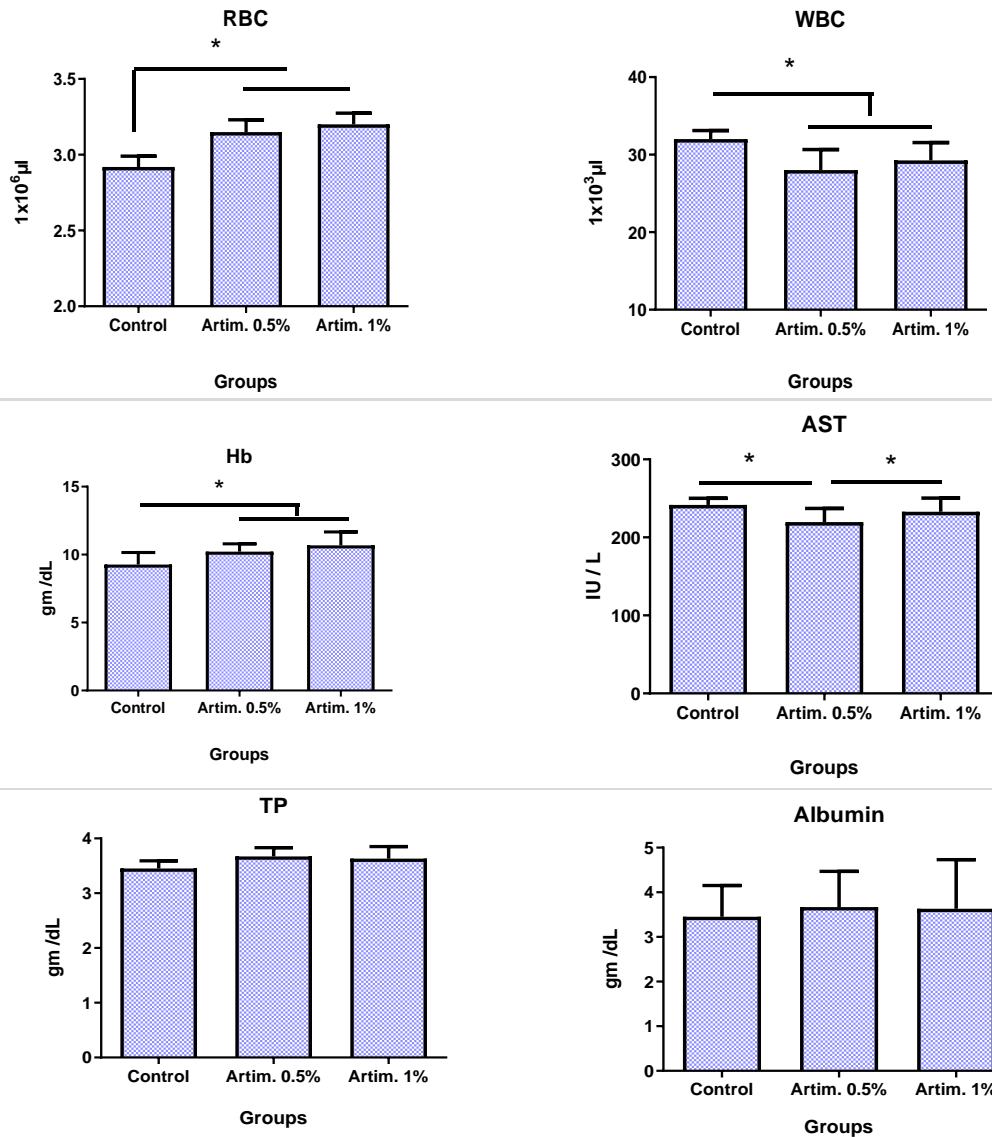
**Table 5.** The relative weight of lymphatic organs of broiler chickens at age 35 days after dietary supplementation with different levels of *Artemisia*

Treatment	Lymphatic organ (%)	
	Spleen	Bursa of Fabricius
G1 (control)	0.06 ± 0.006 <sup>a</sup>	0.08 ± 0.014 <sup>a</sup>
G2 ( <i>Artemisia</i> 0.5%)	0.07 ± 0.006 <sup>a</sup>	0.09 ± 0.012 <sup>a</sup>
G3 ( <i>Artemisia</i> 1%)	0.07 ± 0.007 <sup>a</sup>	0.10 ± 0.012 <sup>a</sup>

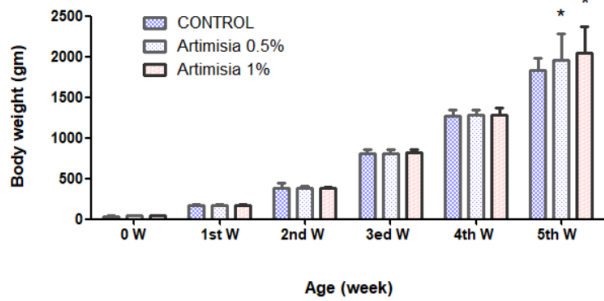
Mean ± standard errors, Alteration in superscript letters in each column refers to a significant difference between treatments at  $p < 0.05$ .



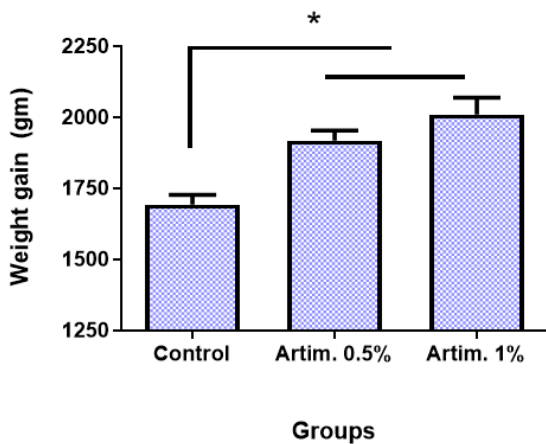
**Figure 1.** The level of antibody titer against Newcastle disease vaccine in broiler chickens aged 35 days after dietary supplementation of different levels of Artemisia



**Figure 2.** The responses of physiological parameters to dietary supplementation of Artemisia in broiler chickens aged 35 days. These are RBCs, WBCs, Hb and AST, TP, and Albumin



**Figure 3.** Body weight of broiler chickens from first to fifth week age after dietary supplementation of different levels of Artemisia.



**Figure 4.** The body weight gain of broiler chickens at 35 days old after dietary supplementation with different levels of Artemisia

## DISCUSSION

The immune response was estimated by measuring antibody titer which was significantly higher in G3, compared to other groups (Figure 1). This significant improvement of antibody titer could be due to the promotion of the dendritic cells and their maturation as a result of increased expression of CD86 and CD40. Consequently, antigen phagocytosis decreases, and the function of T-cells is improved (Wang et al., 2019). Other researchers have reported the enhancement of antibody levels after influenza vaccination, in addition to the improvement in lymphocyte proliferation and cytokine secretion (Zhang et al., 2017). This finding was in line with the one reported by AL-Saedi et al. (2018) indicating an increased level of antibody titer in vaccinated laying hens with Newcastle and Infectious

bronchitis diseases vaccine when fed with Artemisia additive.

The mean of the body weight gains of chickens fed diets supplemented with Artemisia 0.5% and 1% in the current study differed significantly from the control group. This finding indicated that the chickens of the current study could positively benefit from the diet containing Artemisia. Ration containing Artemisia has enhanced the broiler productivity performance throughout the experiment period. The increment of body weight and immunity as a result of a diet containing Artemisia additive could lead to promoting the digestion and absorption of nutrients in the intestine. According to previous studies, Artemisia has abundant bioactive composites, such as coumarin, flavonoids, purines, and phenols (Carrà et al., 2014). The polyphenol substances act as numerous biological activities, among which are anti-inflammatory, antipyretic, anti-cancer, anti-fungal, antiparasitic, and cytotoxic activities (Ćavar et al., 2012). Moreover, it improves gut integrity by increasing villous height and villous to crypt depth (Abdullah and Al-Barwary, 2020). Therefore, dietary supplementation of Artemisia leads to an increased concentration of serum globulin. Liu et al. (2019) have reported that Artemisia enhances IgA levels, and decreases IL-2 and IL-6 in the intestine). Furthermore, it reduces the colonization of pathogenic microbes, such as coccidia, Enterobacteriaceae, *Escherichia coli*, and *Staphylococcus aureus* and improves the viable counts of bacteria-produced lactic acid (Lactobacilli, Saracila et al., 2018; Panaite et al., 2019; Abdullah and Al-Barwary, 2020). All these positive properties of Artemisia have consequently enhanced the immune status and performance.

Moreover, Artemisia enhances the activity of catalase, superoxide dismutase, glutathione peroxidase, and total antioxidant capacity, while reducing the concentration of malondialdehyde in serum, liver, and spleen (Guo et al., 2020). Therefore, improved liver enzymes activities lead to better health conditions.

The results of blood biochemical parameters and cells count of the experiment groups at age of 35 days are in agreement with other studies, such as those conducted by El-Latif et al. (2013) and Oleforuh-Okoleh et al. (2015). As the studies suggested the increment of both RBCs and Hb in broiler chickens as a result of different phyto-genic feed additives. The measurement of blood components could be employed for interpreting the health status (Maxwell et al., 1990). In the current study, it has been noticed that the levels of blood parameters were in normal ranges which could be interpreted as better health and

immune status of the experimental chickens. Nevertheless, the clinical investigations indicated no abnormalities in broiler chickens. The results showed an increase in WBCs count in the control group, compared to the other two experimental groups. On the other hand, the results indicated that the feed additive of 0.5% reduced the level of aspartate aminotransferase, compared to the control group indicating the viability role of *Artemisia* in enhancing liver activities. Reduced activities of the liver enzyme in the current study confirm the appropriate doses of the investigated herbal additive (Sohail et al., 2012; Ali et al., 2014). No significant differences were recorded in both total serum protein and albumin. These findings were similar to those reported in previous studies (Bhaisare and Thyagarajan, 2014). However, enhancements have been noticed in total protein, which could refer to a better immune state of the chickens fed with *Artemisia* additives (Kapelański et al., 2004).

## CONCLUSION

In conclusion, broiler chickens fed a diet containing *Artemisia* powder at a level of 1% for 35 days indicated an improved growth rate and immune response. It is suggested to investigate the effect of adding *Artemisia* to broiler chickens' diet on the immune responses against Gumboro and infectious bronchitis vaccines in extensive research.

## DECLARATION

### Authors' contribution

Haider Kaab wrote the paper and corresponded to complete further requirements of writing. Samer and Ali conducted the experiment. All authors confirmed the statistical results and the final version of the article.

### Acknowledgment

The researchers offer their gratitude to the University of Kufa, Faculty of Veterinary Medicine to help with providing animal houses to conduct the experiment.

### Competing interest

No conflict of interest for this manuscript has been stated by the authors.

### Ethical considerations

The results (or any part of them) used in the manuscript have not been sent for publication to any other journal nor have they already been published. The authors

declare that they have checked the manuscript for plagiarism and there is no data fabrication or redundancy. The performed experiment complied with current laws and written consent of the Scientific Ethical Committee in the department of the pathology and poultry diseases in the College of Veterinary Medicine University of Kufa and Baghdad.

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