

## Original Research

Effect of using water extract of nettle leaves (*Urtica dioica*) on some immunological and blood traits of broiler**Authors:**

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**ABSTRACT:**

The present study aimed at showing the effect of using water extract of Nettle leaves (*Urtica dioica*) on some immunological and blood traits of broiler. 240 chicken broiler (Ross 308) were used at one day aged and 43 g mean weight. They were randomly distributed in four treatments with 60 chick per treatment having three replicates for each. Experimental treatments included: First treatment T<sub>1</sub> Control treatment without any addition of plant extract, the other treatments (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) were added to the water extract of nettle leaves with three concentrations (10, 15, 20 ml/l) in the drinking water, respectively. The results of this study showed significant improvement (P≤0.05) in the immunological traits (Delayed Type Hypersensitivity test (DTH), Enzyme Linked Immunosorbent Assay (ELISA), relative weight of fabricia and fabricia index) and blood Traits (red blood cells, white blood cells, Packed Cell Volume (PCV), hemoglobin concentration and Heterophil/Lymphocyte ratio (H/L) for water extraction of the nettle leaf groups and compared with the control treatment.

**Keywords:**

Immunological, Blood traits, Nettle leaves, Water extracts, Broiler.

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## INTRODUCTION

At present, there is a great competition between international companies specialized in the poultry industry to produce new breeds of broiler, to treat the problems of food shortages in the world quickly and the provision of high-value animal protein (Wepruk and Church, 2003). The production process of broiler has been subjected to many problems, including severe diseases that led to increased use of medical drugs intensively (Siegel, 1995), the disadvantages of these drugs were a high price and had a negative impact on the birds and consumers health (UNESCO, 1992), therefore, the specialists in the trend towards the use of medicinal plants in poultry, substitute for medical drugs, one of these medicinal plants is the nettle plant, Urticaceae family, which includes a range of micro-thorn plants (Al-Rawi and Chakravarty, 1988). The vegetative part of this plant is used to treat many diseases as an integrated pharmacy as well as the importance of the use of feed additives in livestock, especially poultry (Hughes *et al.*, 1980). Therefore, the current study aims to know the effect of the water extract of nettle leaves on some of the immunological and blood characteristics of broilers.

## MATERIALS AND METHODS

This experiment was conducted at the Agricultural Research Station, Agriculture college, Al-Muthanna University from 25.10.2017 to 06.12.2017. 240 chicken broilers which were one day old with an average weight of 43g were used and the chicks were raised in four-story cages, each floor containing a cage of 1.5×1m<sup>2</sup>. Chicks were randomly distributed to four experimental treatments with 60 broilers per treatment. Each treatment included three replicates (20 chick/replicate). The experimental treatments included:

- First treatment (T<sub>1</sub>): Control without addition.
- Second treatment (T<sub>2</sub>): 10 ml/l water extract of nettle leaves were added in the drinking water.
- Third treatment (T<sub>3</sub>): 15 ml/l water extract of nettle leaves were added in the drinking water.
- Fourth treatment (T<sub>4</sub>): 20 ml/l water extract of nettle leaves in were added in the drinking water.

Birds were fed on two types of diets, starter for 1-21 days, containing 21.52% protein and 2994 kcal per kg and finisher for 24-42 days, containing 19% protein and 3141 kcal per kg of energy. The pelleted feed was produced by Al-Hafiz company, Karbala Private, Iraq.

**Table 1. Composition of basal diet**

S. No	Items	Basal Diet	
		1 to 21 d	22 to 42 d
1	Corn	42.0	52.2
2	Wheat	21.4	16.0
3	Soybean meal (44%)	34.0	27.7
4	Mineral and vitamin premix	1.0	1.0
5	Plant oil	0.5	1.8
6	Limestone	0.8	0.6
7	Dicalcium phosphates	0.3	0.3
	<b>Total</b>	<b>100 %</b>	<b>100 %</b>
<b>Calculated analysis</b>			
1	Crude protein (%)	21.52	19.00
2	Metabolizable energy (kilo calorie per kg. Diet)	2994	3141
3	Calcium (%)	0.93	0.85
4	Phosphorus (%)	0.48	0.45
5	Methionine (%)	0.55	0.50
6	Lysine (%)	1.35	1.25
7	Methionine+ cysteine (%)	0.91	0.85
8	Folic acid (%)	1.20	1.10

Calculated analysis according to NRC (1994)

**Table 2. Effect of nettle leaf water extract on the immune response of broilers (mean  $\pm$  standard error)**

S. No	Treatments	DTH (Mm)	ELISA (Titer)	Relative weight of Bursa (%)	Bursa Index
1	T <sub>1</sub>	0.146 $\pm$ 0.001 <sup>c</sup>	2554.5 $\pm$ 3.12 <sup>d</sup>	0.058 $\pm$ 0.001 <sup>d</sup>	1.000 $\pm$ 0 <sup>d</sup>
2	T <sub>2</sub>	0.164 $\pm$ 0.002 <sup>b</sup>	2674.1 $\pm$ 5.12 <sup>c</sup>	0.076 $\pm$ 0.002 <sup>c</sup>	1.322 $\pm$ 0.034 <sup>c</sup>
3	T <sub>3</sub>	0.167 $\pm$ 0.001 <sup>b</sup>	2693.1 $\pm$ 4.34 <sup>b</sup>	0.093 $\pm$ 0.002 <sup>b</sup>	1.603 $\pm$ 0.036 <sup>b</sup>
4	T <sub>4</sub>	0.175 $\pm$ 0.001 <sup>a</sup>	2760.6 $\pm$ 8.73 <sup>a</sup>	0.108 $\pm$ 0.002 <sup>a</sup>	1.862 $\pm$ 0.045 <sup>a</sup>
5	Significance	*	*	*	*

T<sub>1</sub>: Control; T<sub>2</sub>: added 10 ml/l water extract of nettle leaves in drinking water; T<sub>3</sub>: added 20 ml/l water extract of nettle leaves in drinking water; T<sub>4</sub>: added 30 ml/l water extract of nettle leaves in drinking water; \*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.05).

Table 1 shows the chemical composition of diet used in the experiment.

Dried leaves were obtained from the local markets and cleaned for removing impurities, then grinded by electric grinder to become a powder, mix 1g dried powder with 1ml distilled water, using the electric mixer, the solution was placed in a water bath at 55°C for one hour. The solution was left undisturbed for 24 hours at room temperature. After extracting the resultant supernatant by filter paper, the extract is made ready for experiment (Hernandez-Pérez *et al.*, 1994).

At the end of the experimental period, two birds per replicate were slaughtered and blood was collected in heparinized tube and centrifuged at 2000 rpm for 15 min. Serum was isolated and stored at 20°C. The indirect Immunosorbent scale was used through an enzyme-related assay (ELISA) to determine the serum level against NDV (Feng *et al.*, 2009). The antigen was injected with 0.1 ml of reaction mixture in the skin of the right comb (Intradermal). The left comb was injected with 0.1% formalin saline solution (0.1 ml). It was measured 24 hours after injection, calculated according to the following formula:

$$DTH = \frac{\text{Right comb thickness} - \text{Left comb thickness}}{\text{Left comb thickness}}$$

The weight of bursa and bursa index were measured for each bird. Blood samples were collected at the end of the sixth week, from vein brachial of six birds for each treatment, with 10 ml glass tubes that does not contain anticoagulation. The number of red and white blood cells and packed cell volume were calculated.

Other blood samples were centrifuged at 3000 rpm/min for 15 minutes, to measure proteins, hemoglobin concentration, Heterophil/Lymphocyte cell ratio (H/L) values. The analysis was conducted in Al-Rehab Analysis Lab in Baghdad, Iraq.

Data generated from the present experiment was subjected to statistical analysis using the GLM procedure of Statistical Software Package SAS (2001). When significant differences were noted, mean were compared using Duncan's multiple range test (1955).

## RESULTS

### Immunological traits

Table 2 shows the effect of the use of the water extract of nettle leaves in the immune response of broiler. The results showed that DTH had a significant increase ( $P \leq 0.05$ ) in T<sub>4</sub> compared with T<sub>2</sub> and T<sub>3</sub>, While T<sub>2</sub> and T<sub>3</sub> treatments have significantly increased ( $P \leq 0.05$ ) compared with the control treatment. No significant differences were seen between T<sub>2</sub> and T<sub>3</sub>. Other immunological characteristics (ELISA, bursa relative weight and bursa index) showed a significant increase ( $P \leq 0.05$ ) of T<sub>4</sub> compared to T<sub>3</sub> and T<sub>3</sub> showed a significant increase ( $P \leq 0.05$ ) compared with the T<sub>2</sub>, while T<sub>2</sub> was significantly higher ( $P \leq 0.05$ ) than the control treatment.

### Blood traits

Table 3 shows the effect of the use of water extract of nettle leaves on some blood traits of broiler, the results of some of blood traits (RBC, WBC, PCV, Hb and globulin concentration) of broiler showed a signifi-

**Table 3. Effect of nettle leaf water extract on the blood traits of broilers (mean± standard error)**

Treatments	Cellular blood traits			Blood plasma proteins				H/L
	RBC (10 <sup>9</sup> /ml)	WBC (10 <sup>3</sup> /ml)	PCV (%)	Hb (gr/dl)	Albumin (mr/dl)	Globulin (mr/dl)	Transferrin (mr/dl)	
T <sub>1</sub>	2.68±0.017 <sup>d</sup>	28.95±0.092 <sup>d</sup>	40.98±0.019 <sup>d</sup>	14.02±0.107 <sup>d</sup>	2.21±0.011 <sup>b</sup>	2.09±0.034 <sup>d</sup>	9.13±0.015	0.22±0.003 <sup>c</sup>
T <sub>2</sub>	2.79±0.008 <sup>c</sup>	29.26±0.032 <sup>c</sup>	42.26±0.094 <sup>c</sup>	14.44±0.062 <sup>c</sup>	2.40±0.008 <sup>a</sup>	2.20±0.005 <sup>c</sup>	9.13±0.015	0.26±0.017 <sup>b</sup>
T <sub>3</sub>	2.89±0.008 <sup>b</sup>	29.51±0.024 <sup>b</sup>	43.91±0.047 <sup>b</sup>	14.73±0.025 <sup>b</sup>	2.49±0.005 <sup>a</sup>	2.42±0.025 <sup>b</sup>	9.13±0.012	0.30±0.008 <sup>a</sup>
T <sub>4</sub>	2.98±0.002 <sup>a</sup>	29.72±0.020 <sup>a</sup>	44.60±0.352 <sup>a</sup>	15.00±0.031 <sup>a</sup>	2.48±0.069 <sup>a</sup>	2.71±0.008 <sup>a</sup>	9.10±0.050	0.27±0.005 <sup>ab</sup>
Sig.	*	*	*	*	*	*	N.S	*

T<sub>1</sub>: Control; T<sub>2</sub>: added 10 ml/l water extract of nettle leaves in drinking water; T<sub>3</sub>: added 20 ml/l water extract of nettle leaves in drinking water; T<sub>4</sub>: added 30 ml/l water extract of nettle leaves in drinking water; \*Different letters vertically indicate the existence of significant differences between the averages at the possibility of (0.05); NS: Non-significant.

cant increase ( $P \leq 0.05$ ) of T<sub>4</sub> compared to T<sub>3</sub>, T<sub>3</sub> was significantly increased ( $P \leq 0.05$ ) compare with T<sub>2</sub>, while T<sub>2</sub> was significantly higher ( $P \leq 0.05$ ) than the control treatment. A significant increase ( $P \leq 0.05$ ) of all of the water extract of nettle leaves treatments were compared with the control treatments on the albumin concentration. There were no significant differences between all the extracts treatment. Result of the Heterophil/Lymphocyte cell ratio (H/L) values showed that a significant increase ( $P \leq 0.05$ ) of all of water extract was compared with the control treatment. A significant increase ( $P \leq 0.05$ ) on T<sub>3</sub> was compared with T<sub>2</sub>, there were no significant differences between T<sub>2</sub> and T<sub>4</sub>, T<sub>3</sub> and T<sub>4</sub>.

## DISCUSSION

### Immunological traits

This significant improvement in the immune response to the addition of the water extract may be attributed to the effectiveness of both flavonoids and phenols in increasing the activity of the immune system, by raising the level of antibodies directed against pathogens as well as by the increase of white blood cells and the immunoglobulin in the blood (Fischer *et al.*, 2007; Galal *et al.*, 2008). The saponins found in the nettle leaves were important in supporting the immune system by forming a series of T-type lymphocytes, which are produced by the lymphocytes, responsible for the activation of inflammatory cells against pathogenic microbes (Abouhosseini *et al.*, 2016). Tannins have an antimicrobial role, leading to the increased immuno-

globulin and thus positively reflected on the overall health of the birds (Mehdi *et al.*, 2014).

### Blood traits

The improvement in some blood traits (RBC, WBC, Hb, and PCV) of the water extract of the nettle leaves compared to the control treatment, was due to the vitamins and minerals such as iron and copper found in nettle leaves, preservation of the cells membranes from oxidation with less degradation, leading to the increased PCV, and Hb concentration (Mansoub, 2011). Red Blood Cells number (RBC) increased causes an increase in PCV and hemoglobin concentration. Previous studies show that the addition of nettle powder to diet caused improvement in the immunity by albumin and serum responsible for building the muscle structure of broiler. In addition, there were no significant difference in transferrin, because it rises in cases of severe inflammation as an immune protein is not specialized (Safamehr *et al.*, 2013). The improvement in the ratio of heterophil to lymphocyte cells in the water extract treatments could be attributed to an increase in the number of T lymphocytes increasing as a result of the increased immune response (Hyrb *et al.*, 1995).

## CONCLUSION

The water extract of nettle leaves contributed to the improvement of the immune response as well as some of the characteristics of the meat, high levels of water extract of 20 ml/liter of drinking water have given the best results compared to other levels of water extract

(10 and 20 ml/l of drinking water).

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