



## Improving Behavior, Immune Status and Growth of Broilers by Adding Some Neurotransmitters in Drinking Water and Hero Flowers (*Alcea Kurdica*) in Diets

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**T**HE role and action of the active chemical compounds and native flowers to improve immunity system function and behavior in the broiler. This study has investigated the impact of adding some neurotransmitters (Endorphin, Dopamine, and Serotonin) in drinking water and native Hero flowers (*Alcea kurdica*) in diets on behavior, immune status, and growth of broilers (Ross-308). 720 unsexed hatched chicks will be distributed into 8 groups; T0 (control -standard diet), [T1 (0.2 gr endorphin), T2 (0.2 gr dopamine) and T3 (0.2 gr serotonin)/ L drinking water], [T4 (0.5 kg Hero flower (Hf))/100 kg diet], T5 (0.2 gr endorphin + 0.5 kg Hf), T6 (0.2 gr dopamine + 0.5 kg Hf) , T7 (0.2 gr serotonin+ 0.5 kg Hf) . The results of neurotransmitters and Hf additives led to a significant ( $P \leq 0.01$ ) increase in eating behavior when birds were in an up position, wing flapping, dust bathing, walking and preening, serum concentrations of endorphin, dopamine, serotonin, immunoglobins IgG, IgA, IgM, and antibodies titer against Newcastle (ND), and Infectious Bronchitis Viral (IBV) diseases compared with control T0, improved histological of Bursa, body weight, body weight gain, improved feed conversion ratio (FCR), and pathohistology bursa of Fabricius , and feasibility. While the results of eating when birds were at set position, immobility, pecking, feather pecking, aggressiveness and mortality percentages were decreased.

**Keywords:** Neurotransmitters, Native Flower, Broiler, Behavior, Immune Statue.

### Introduction

The immune system of the poultry is very strong in preventing diseases and helping to ensure maximum production performance [1]. Neurotransmitters are chemical messengers for conveying chemical signals from one neuron (nerve cell) to the next target cell, another nerve cell, a muscle cell, or a gland might be the next target cell and play a vital role in appetite regulation, cognition, locomotor activity, food intake, behavior regulation, and many other physiological functions in birds [2]. Dopamine is one of the major neurotransmitters in the central nervous system known as "catecholamines" for their pharmacological characteristics. Dopamine has been revealed to activate T cell function. And also modulate suppresses immune functions. Endorphins are natural painkillers and polypeptides released as a reaction to pain or stress, and serotonin supports

feelings of well-being primarily by regulating mood, appetite, sleep cycles, locomotion, and feeding [3- 5].

Poultry phasing causes several problems during growth periods, that affect the broiler's growth, performance, and behavior. Many researchers turned to use medicinal plants such as (*Alcea kurdica*) (local name: Hero flowers) which belongs to the family Malvaceae, is a polymorphic and commonly dispersed species found in east Iraq and west Iran, Hf is a major source of mucilage, and there are roughly 70 different species, originate in the Erbil, Sulaimaniyah, Kirkuk, Sallahaddin, and Mosul, etc. [6]. Also, Hf used as an antioxidant, antimicrobial [7], and as veterinary medicine to treat carminative coughs and infusions [6]. Also, Hf is used in diets to increase broiler appetites, and stimulate production performance [8], and the center of appetite in the brain to increase feed

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consumption, stimulate the digestion process, enhance immunity, natural antioxidants, and the active chemicals in Hf are sesquiterpenes, flavonoids, polyacetylenes, and phenolic compounds [8-10]. This study aimed to compare the beneficial effects of these additives (Dopamine, Endorphins, Serotonin, and Hf) to choose the best one concerning the behavior and immune status of broiler chickens.

## **Material and Methods**

### **Study Area and Experimental Design:**

This study was conducted in the poultry hall in the farm of Grdarasha, Animal Resources Dept., College of Agricultural Engineering Sciences, University of Salahaddin- Erbil, Iraq. One day 720 unsexed hatched chicks reared for 42 days and distributed into 12 groups: (60 chicks for a treatment distributed into three replicates, 20 chicks for each replicate), T0 (control (standard diet), T1 (0.2 gr Endorphin), T2 (0.2 gr dopamine) and T3 (0.2 gr serotonin)/ L drinking water], [T4 (0.5 kg Hf/ 100 kg diet], T5 (0.2 gr endorphin + 0.5 kg Hf), T6 (0.2 gr dopamine + 0.5 kg f), T7 (0.2 gr serotonin+ 0.5 kg Hf). Standard feed and water were served *ad libitum*.

The experimental feeds were prepared in Evan Company and contained starter (1-10), grower (11-25), and finisher (26-42) days: 3097, 2990, and 2906 kcal/kg metabolizable energy, and 22.97, 21.50, and 20.78 % crude protein respectively. Chicks received 24 hours of light (L) during the 1<sup>st</sup> week. Then gradually decreased to a 20 L:4 D schedule. Also, birds were vaccinated against Gamboro at 7 and 14 days and Newcastle at 1, 14, and 28 days of rearing. The chemical analysis of total antioxidant (116.05) mg/100 gr, phenolic (171.24) mg/100 gr, and flavonoid (1140.5) µg CE/gr, respectively. Compounds in Hf were determined in this study by High-performance liquid chromatography (HPLC).

**Behavioral characteristics:** Behavioral were recorded through observation of broiler chickens housed by the installation camera at different groups (T0-T7), parameter description recording (agonistic behavior, preening, dust bathing, scratching, pecking, feather pecking, walking, wing flapping, immobility, drinking, eating) at age 42 days.

**Blood samples:** Blood samples were taken after slaughtering birds at 9:00 AM, at the end of 42 d old, then serum was prepared and determined by using ELISA according to the instructions of the kit involved in the Buyer's Guide for Life Science Bio-

compare, correspondingly to [11], antibodies titer against ND and IBV diseases according to Abdel Moneim *et al.* [12] by using the Indicial Bioscience kit, and immunoglobins IgA, IgM and IgG concentrations were determined by ELISA to evaluate the immune response of chickens against T-independent antigens by ELISA, according to Abdel Moneim *et al.* [13], the absorbance was read at 450 nm (Microplate reader Bio-Compare, USA).

**Immunoglobins:** Immunoglobulins (IgG, IgA and IgM) were determined as described by kits from Wondfo Biotech Co. Ltd.

**Body growth:** Body weight, weight gain, feed intake, Feed Conversion Ratio (FCR), mortality and feasibility were measured during 42 days according to Olarotimi, and Adu [14].

**Histology of some immune organs:** Bursa samples taken from the chicks of all groups at age 42 days succumbed for histological testing. A tissue section of these organs was removed and fixed in 10% neutral buffered formalin. All tissue samples were fixed in paraffin wax, segmented at 5µm, then passed on clean glass slides and stained routinely with Hematoxylin and Eosin stains for detection of histological changes [15, 16].

### **Statistical analysis:**

All data were analyzed according to CRD (Complete Randomize Design) by the SAS (17) program, as per variance, significant differences among treatment means were determined by using one Way analysis of variance ANOVA, Duncan test; comparing all groups against control group at levels  $P \leq 0.01$  and  $P \leq 0.05$ .

## **Results**

### **Broiler behavioral traits and neurotransmitter levels in serum**

Tables 1 and 2 show that adding neurotransmitters (endorphin, dopamine, and serotonin) to drinking water and native additives of Hf and their use together had significantly ( $P \leq 0.01$ ) increased eating behavior when birds were in the up position, wing flapping, dust bathing and preening, also significantly ( $P \leq 0.05$ ) increased walking in additive groups, especially in T6 and T7 compared with T0. However, the results show a significant ( $P \leq 0.01$ ) decrease in eating when birds were at a set position, Immobility, pecking, feather pecking, and aggressiveness, also significant ( $P \leq 0.01$ ) lower in water drinking behavior when birds were in the up position in additive groups compared with control T0.

**TABLE 1.** The impact of adding neurotransmitters in drinking water and Hf in diets on eating, drinking and aggressiveness behaviors of broiler at age 42 days.

Groups	Eating behavior %		Drinking behavior %		Aggressiveness %
	Set	Up	Set	Up	
T0	7.67 <sup>a</sup>	10.40 <sup>d</sup>	4.45 <sup>a</sup>	19.65 <sup>a</sup>	4.13 <sup>a</sup>
T1	5.72 <sup>b</sup>	13.48 <sup>c</sup>	3.80 <sup>b</sup>	19.40 <sup>a</sup>	1.35 <sup>b</sup>
T2	5.48 <sup>bc</sup>	15.06 <sup>ab</sup>	3.33 <sup>b</sup>	17.78 <sup>b</sup>	1.11 <sup>b</sup>
T3	4.40 <sup>c</sup>	15.44 <sup>a</sup>	3.38 <sup>b</sup>	17.55 <sup>b</sup>	0.99 <sup>bc</sup>
T4	4.91 <sup>c</sup>	15.61 <sup>a</sup>	2.82 <sup>bc</sup>	17.81 <sup>b</sup>	0.73 <sup>c</sup>
T5	4.94 <sup>c</sup>	13.41 <sup>c</sup>	2.82 <sup>bc</sup>	17.35 <sup>bc</sup>	0.84 <sup>c</sup>
T6	5.36 <sup>bc</sup>	14.93 <sup>b</sup>	2.59 <sup>bc</sup>	16.77 <sup>c</sup>	0.69 <sup>c</sup>
T7	5.15 <sup>c</sup>	15.77 <sup>a</sup>	2.35 <sup>c</sup>	16.58 <sup>c</sup>	0.66 <sup>c</sup>
SEM	0.392	0.947	0.108	1.264	0.092

**TABLE 2.** The impact of adding neurotransmitters in drinking water and Hf in diets on broiler behavior traits at age 42 days.

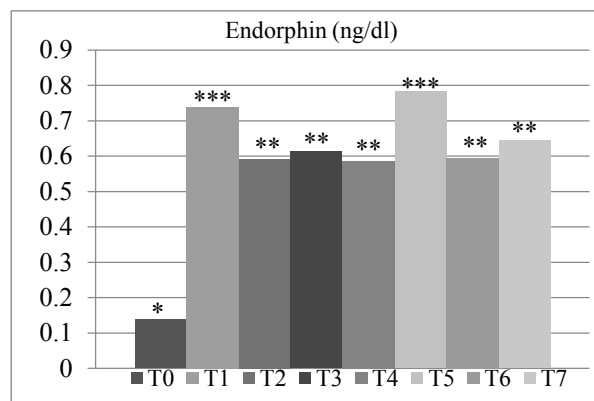
Treatments	Broiler Behaviours %						
	Walking	Immobility	Wing flapping	Dust bathing	Preening	Pecking	Feather pecking
T0	6.62 <sup>d</sup>	21.75 <sup>a</sup>	3.15 <sup>c</sup>	4.29 <sup>c</sup>	6.34 <sup>c</sup>	6.22 <sup>a</sup>	5.33 <sup>a</sup>
T1	7.11 <sup>cd</sup>	21.03 <sup>ab</sup>	4.27 <sup>b</sup>	6.37 <sup>b</sup>	9.45 <sup>b</sup>	5.53 <sup>b</sup>	2.49 <sup>b</sup>
T2	7.56 <sup>c</sup>	20.38 <sup>b</sup>	5.06 <sup>ab</sup>	7.08 <sup>a</sup>	9.76 <sup>ab</sup>	5.35 <sup>b</sup>	2.05 <sup>b</sup>
T3	9.35 <sup>b</sup>	20.05 <sup>b</sup>	5.40 <sup>ab</sup>	7.73 <sup>a</sup>	9.53 <sup>b</sup>	5.27 <sup>b</sup>	0.91 <sup>c</sup>
T4	8.99 <sup>b</sup>	19.52 <sup>bc</sup>	5.26 <sup>ab</sup>	6.82 <sup>ab</sup>	10.20 <sup>a</sup>	5.48 <sup>b</sup>	1.85 <sup>bc</sup>
T5	10.02 <sup>ab</sup>	19.80 <sup>bc</sup>	5.92 <sup>a</sup>	6.89 <sup>ab</sup>	10.23 <sup>a</sup>	5.59 <sup>b</sup>	2.19 <sup>b</sup>
T6	10.47 <sup>a</sup>	19.11 <sup>bc</sup>	5.89 <sup>a</sup>	7.60 <sup>a</sup>	10.29 <sup>a</sup>	5.21 <sup>b</sup>	1.09 <sup>c</sup>
T7	10.50 <sup>a</sup>	18.92 <sup>c</sup>	5.85 <sup>a</sup>	7.68 <sup>a</sup>	10.52 <sup>a</sup>	5.02 <sup>b</sup>	1.00 <sup>c</sup>
SEM	0.377	1.769	0.233	0.280	0.402	0.475	0.109

a, b, c, d, mean values within the same columns were significantly different (P≤0.01).

**Neurotransmitters concentrations in blood serum:**

Figures 1, 2, and 3. show that the addition of neurotransmitters (dopamine, endorphin, and serotonin) to drinking water and native Hf, and its use with neurotransmitters) to broiler had a

significant (P≤0.01) impact, it increased serum concentration of endorphin, dopamine, and serotonin, especially endorphin in T1 and T5, dopamine in T2 and T6, serotonin in T3 and T7 compared with control T0.



**Fig. 1.** Serum concentration of Endorphin (ng/dl)

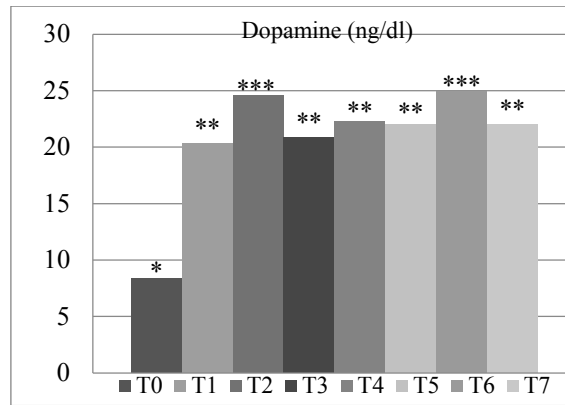


Fig. 2. Serum concentration of Dopamine (ng/dl)

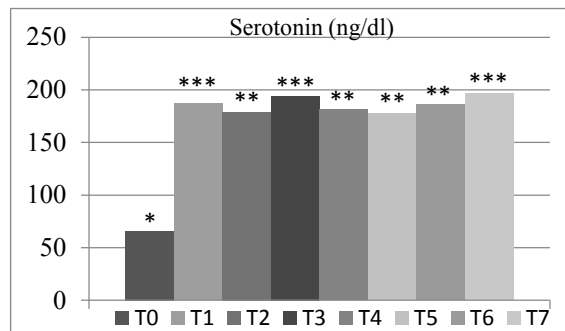


Fig. 3. Serum concentration of Serotonin (ng/dl)

**Immunoglobins concentrations in blood serum:**

Figure 4. shows that the addition of neurotransmitters and native flower additives, and their use together as immune modulators led to a significant increase ( $P \leq 0.01$ ) in IgG, IgA and IgM

concentrations in all additive treatments compared with the control treatment T0, especially IgG in T5, T6 and T7, IgA in T3 and T7), and IgM in T7.

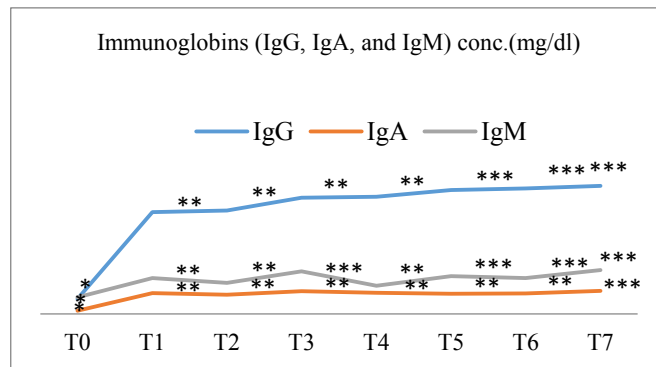


Fig. 4. Immunoglobins (IgG, IgA, and IgM) concentrations (mg/dl) in blood serum

The significance results were considered at \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.01$ , \*\*\*\*  $p \leq 0.01$ .

**Antibodies titer against ND and IBV disease:**

It is noted from Table 5. That the antibodies titer at age 42 days against ND and IBV diseases had significantly ( $P \leq 0.01$ ) increased in all the

treatments of adding endorphin, dopamine, and serotonin in water and Hero flowers in diets compared with the control T0, especially ND in the treatments T6 and T7, also IBV in T5, T6 and T7.

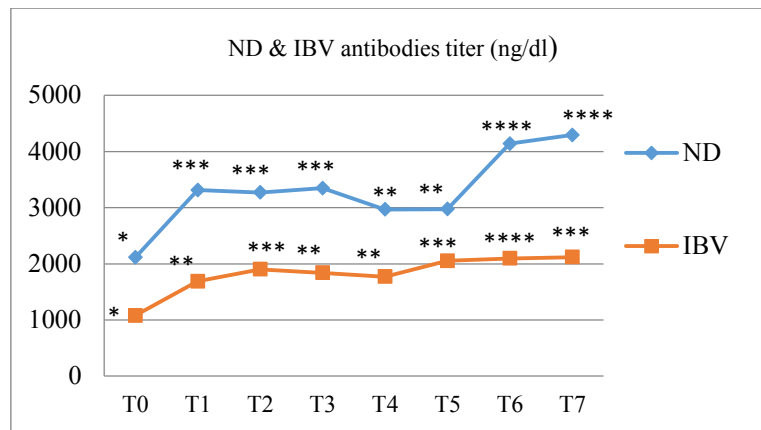


Fig. 5. ND & IBV antibodies titer (ng/dl)

According to data in Table 3. the impact of adding neurotransmitters (dopamine, endorphin, and serotonin) in drinking water, and Hf in diets and using them together had significantly ( $P \leq 0.01$ ) increased body weight, body weight gain, and feasibility %, also improved in feed conversion ratio (FCR), While, significant ( $P \leq 0.01$ ) lower

mortality when compared with the control group. However, didn't see any significant differences among the additive groups and control in feed intake. In all traits, the groups of adding HF in the diet together with serotonin (T7) or dopamine (T6) achieved superior results.

TABLE 3. The impact of adding neurotransmitters in broiler drinking water and Hero flowers in diets broiler on body performance at 42 days.

Treatments	BW (g)	BWG (g)	FI (g)	FCR	Mortality (%)	Feasibility \$/kg
T0	2900 <sup>c</sup>	2858.2 <sup>c</sup>	5101.9 <sup>a</sup>	1.785 <sup>a</sup>	5.8 <sup>a</sup>	0.57 <sup>c</sup>
T1	3150 <sup>b</sup>	3108.2 <sup>b</sup>	5386.5 <sup>a</sup>	1.715 <sup>b</sup>	2.5 <sup>b</sup>	0.68 <sup>b</sup>
T2	3225 <sup>ab</sup>	3183.2 <sup>a</sup>	5516.5 <sup>a</sup>	1.733 <sup>b</sup>	2.5 <sup>b</sup>	0.69 <sup>b</sup>
T3	3319 <sup>a</sup>	3277.2 <sup>ab</sup>	5030.5 <sup>a</sup>	1.535 <sup>d</sup>	1.5 <sup>cd</sup>	0.71 <sup>ab</sup>
T4	3275 <sup>a</sup>	3033.2 <sup>b</sup>	5165.5 <sup>a</sup>	1.703 <sup>bc</sup>	1.5 <sup>cd</sup>	0.73 <sup>ab</sup>
T5	3205 <sup>ab</sup>	3163.2 <sup>ab</sup>	5279.4 <sup>a</sup>	1.669 <sup>bc</sup>	2.0 <sup>c</sup>	0.73 <sup>ab</sup>
T6	3275 <sup>a</sup>	3233.2 <sup>a</sup>	5334.8 <sup>a</sup>	1.650 <sup>bc</sup>	1.9 <sup>c</sup>	0.74 <sup>ab</sup>
T7	3375 <sup>a</sup>	3333.2 <sup>a</sup>	5359.8 <sup>a</sup>	1.608 <sup>c</sup>	1.0 <sup>d</sup>	0.82 <sup>a</sup>
MSE	311	177	269	0.157	0.50	0.11

a, b, c, d Mean values within the same row were significantly different ( $P \leq 0.01$ ).

### Histological Examination of Bursa Fabricius (BF) organ:

Bursa of Fabricius (BF) is a primary lymphoid organ in poultry, with histomorphometric and microscopic changes of BF collected from broiler chickens. Figure 6. refers to the effect of adding endorphin, dopamine, and serotonin to drinking water and Hf to diet (normal state groups), lymphocyte numbers are obviously increased in lymphoid follicles with thinner cortices and wider medullae, bursal follicles appeared large and densely populated, the cortex was separated from the medulla by a basal membrane, visible in most sections appearance of many cysts and most sub-

epithelial connective tissue compared with the control group T0 which is decreased in the lymphocyte numbers, bursal follicles appeared small and loosely populated, and the cortex was not separated well. As well as, improved histologically BF content of cysts (Cy), the Follicle of bursa (FB) increased in numbers, and plicae are larger in size, height and thickness, also connective tissue (CT) more developed in T4, T6, T7, T1 and T2, T3 respectively compared with T5 and the control T0 which is generally less than the normal in BF content of Cy, the FB and decreased in numbers, and plicae are smaller in size, height and thickness, also the CT is less developed.

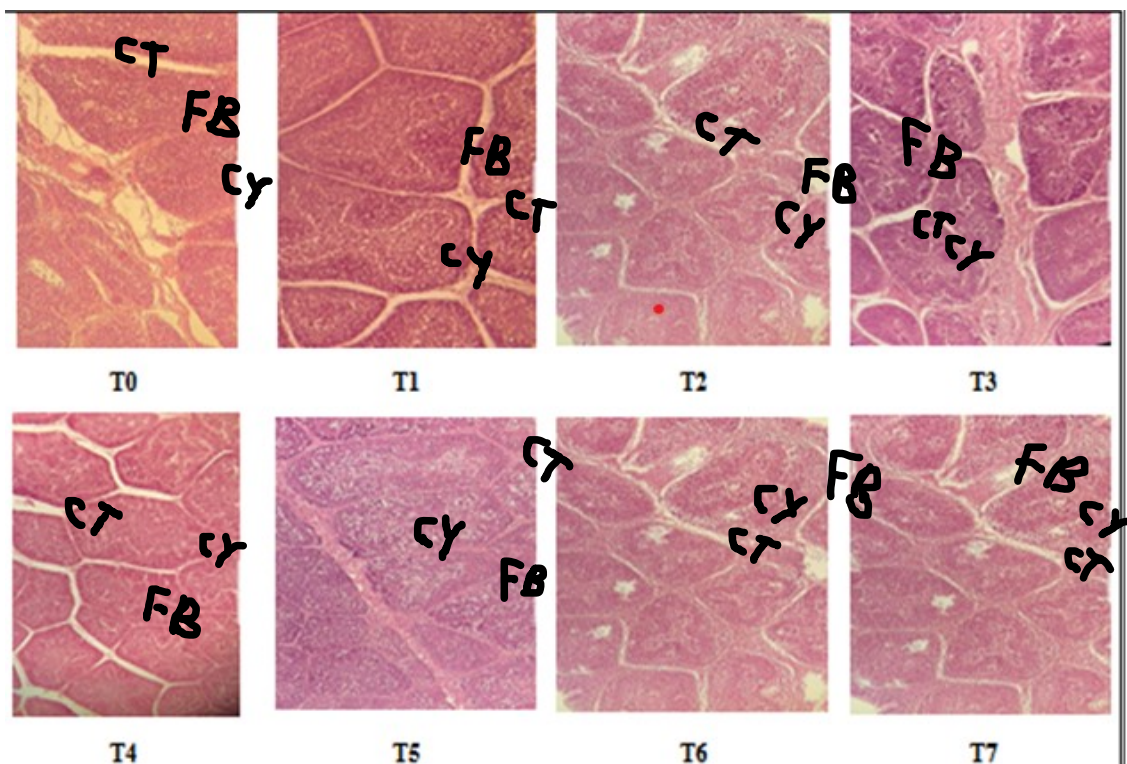


Fig. 6. The impact of adding neurotransmitters in broiler drinking water and Hero flowers in diets of broiler on histopathological of Bursa of Fabricius organ at age 42 days.  
Cy: Cyst, FB: Follicle of bursa, CT: Connective tissue.

### Discussions

The use of medicinal flower extracts is very important as feed additives for appetite stimulators, and immune enhancements [18]. Neurotransmitters mainly support feelings of well-being by regulating mood, appetite, sleep cycles [19], feeding [20], and locomotion [21], it may be beneficial to provide the diet with an optimal concentration of neurotransmitters, especially serotonin and functional feed additives to improve the positive behavioral characteristics that are more important in all body functions, such as the feeling of happiness and good welfare, lower aggressiveness in treated birds [22, 11]. The serotonergic interplay in animal personality would benefit from further studies, especially on the role of serotonin in personality traits such as exploration and sociability to obtain a fuller picture of how serotonin can affect more personalities [23].

In broilers, the immune system plays an important role in maintaining health and preventing diseases. A hygienic immune system can help broilers resist and fight infections, which can cause illness and reduce their growth rate. Hence, making a balance between the growth rate and activity of the immune system is very requested for optimal poultry production [24]. Higher antibody titers against ND, IBD, and IB viruses in the birds was

observed in birds treated with Hf [8], also agree with Fernandes et al. [25], who discovered supplementing feed additives such as herbs and spices or their extracts led to stimulating the appetite. The improvement of broiler behavior may be associated with an increasing concentration of neurotransmitters such as dopamine and serotonin in the additive groups, Which have chemical effects on the brain by improving mood, eating foods, improving overall health and happiness, associated with it, and having a positive impact on the central nervous system [3, 4]. The immune system uses a diversity of mechanisms to accomplish this goal, including inactivation of biological managers, lysis (rupture) of foreign cells, agglutination (clumping) or precipitation of particles or cells, and phagocytosis (engulfing and deactivating) of foreign causes, the immune response has two ways of dealing with foreign pathogens.

The B-lymphocytes produce specific antibodies called immune-globulin; this is recognized as humoral immunity. The other system embraces T-lymphocytes, which control the synthesis of antibodies as well as direct killer cell activity and the inflammatory reaction of delayed-type hypersensitivity [26].

Herbal medicinal drugs have been given to poultry such as broilers, layer, quails, ducks, and pet birds as a feed additive, and daily the solution

of herbs by drinking water to give a positive response for the birds' better progress (low mortality, rare illness) has been reduced [27]. Race broilers and local poultry have all been fed a combination of medicinal herbs as a feed addition, representing enhanced feed efficiency and animal health, there is also a growing consideration of herbal medicines' anti-parasitic potential [28], and according to [29] medicinal herbs support to cope illnesses by depressing stress and oxidative stress, resulting in better nutrition, better health, and increased feasibility. Plants, contrasting mammals, lack mobile protector cells and a somatic adaptive immune system, instead, they rely on the innate immunity of each cell and systemic signals emanating from infection sites, and this type of defense response is due to the presence of a large, diverse array of organic compounds that appear to have no direct function in growth and development, these substances are known as secondary products, or natural products many such compounds occur in nature as anti-infecting chemicals, and are found effective against microbes [30].

The feed additives have components minimizing free radicals raising the antioxidant capacity of the blood and improving the body health of poultry that may enhance immune status and improve birds' immune status, thus these additives may stimulate cells, increasing antibody production and fighting ability of macrophage cells while enhancing the activity of T cells, which are responsible for cellular immunity [31].

From the results, the valuable contents of Hero flowers that confirmed in substantial and methods in the present study, a total antioxidant capability, phenolic, and flavonoid, had effective part in refining body growth and immune status characterized by immune organs and antibodies titer against ND and IBV diseases, may be rising of antioxidant capability due to reductions of free radicals and reactive oxygen species (ROS) in biology is constructing a medical uprising that capacities a new age of health and disease administration [32]. Flavonoids, Alkaloids, and hydroxylated phenols, for example, are naturally produced by plants in response to contamination and flavones, being bitter, also have natural anti-feedant special effects, more recently, many secondary metabolites have been recommended to have immune-modulation properties in animals [27].

### Conclusions

The addition of neurotransmitters (dopamine, endorphin, and serotonin) in drinking water and native flower additives of Hero and its use together had significantly improved behavioral characteristics, neurotransmitters (dopamine and endorphin, serotonin) levels in serum, has clear

effects on the immunity of the bird's body and enhances its health represented by antibodies titer against ND and IBV diseases, also immunoglobulin concentrations (IgG, IgA and IgM) in blood serum, histological improvement Bursa of Fabricius organ, and decreased aggressive, negative behaviors and mortality percentages in all additive groups which enhanced to increasing body weight and gain, and improved FCR, also increased the feasibility (economic profit) of reared broilers.

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## تحسين السلوك، الحالة المناعية وصفات النمو لفروج اللحم بإضافة الناقلات العصبية في ماء الشرب و ازهار الخطمية الى العليقة

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أن لدور وفاعلية اضافة المركبات الكيميائية والأزهار المحلية هو لتحسين وظيفة الجهاز المناعي و سلوك فروج اللحم. هدفت هذه الدراسة إلى تأثير بعض الناقلات العصبية في مياه الشرب بعض الأزهار المحلية كإضافات غذائية على السلوك والصفات المناعية للفروج (Ross-308). وزعت 720 فرخة غير مجنسة بعمر يوم واحد الى 12 معاملة: T0 (عليقة قياسية) ، [ T1 (0.2 غم إندورفين) ، T2 (0.2 غم دوبامين) و T3 (0.2 غم سيروتونين)]/لتر ماء شرب) ، [ T4 (0.5 كغم زهرة الخطمية) و T5 (0.2 غم إندورفين+ 0.5 كغم زهرة الخطمية) ، T6 (0.2 غم دوبامين+ 0.5 كغم زهرة الخطمية) ، T7 (0.2 غم سيروتونين+ 0.5 كغم زهرة الخطمية). أدت إضافة الناقلات العصبية والخطمية المحلية الى ارتفاع معنوي ( $P \leq 0.01$ ) في سلوك تناول العلف عند الطيور في وضع الوقوف، رفرقة الأجنحة، والاستحمام بالغبار، والمشي والتنظيف، كذلك في المعيار الحجمي ضد الأجسام المضادة لأمراض النيوكاسل (ND) والتهاب الشعب الهوائية المعدية (IBV) ، تركيز الكلوبولينات المناعية IgG و IgA و IgM، وزن الجسم، الزيادة الوزنية، الربح الاقتصادي، تحسن كفاءة التحويل الغذائي والتشريح النسيجي لغدة الفابريشيا في جميع معاملات الإضافات مقارنة بمعاملة السيطرة. وانخفض تناول العلف عندما كان الطيور في وضع الجلوس، وعدم الحركة، والنقر، وتنف الريش والعذوانية ونسبة الهلاكات.

**الكلمات المفتاحية:** الناقلات العصبية ، زهور محلية ، فروج اللحم ، السلوك ، الحالة المناعية