THE EFFECTIVENESS OF SOME MEDICINAL PLANTS ON BODY PERFORMANCE, HEMATOLOGICAL, ILEUM MORPHOLOGY AND IMMUNE STATUE OF JAPANESE QUAIL

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ABSTRACT

This study was aimed to investigates and compares the effect of medicinal plants extract: Hero flowers (*Alcea kurdica*) in the north of Iraq, Thyme (*Thymus bovie*), and Bay leaf (*Laurus nobilis*) extract with two antibiotic drugs Rifaximine and Ranitidine in quail's drinking water by emphasizing the productive performance, and physiological status of the quails at grower and developer stages till 10th weeks of age, a total of 90 quails were distributed into six treatments, each treatment with three replicates of 15 chicks in each. Data analysis showed significantly increased body weight gain (237.5, 224.1, and 234.9) g for Hero, thyme, and Bay leaf, respectively. In comparison with the control (188.3) g. quail's drunk plant extract mixed water had better hematological and the ileum profiling (goblet cells numbers, Villus height, and Crypt depth) and significantly higher serum concentrations of total protein, globulin, and antibodies titers against some bird virus diseases in comparison with positive control. Also, significantly lower level of albumin, ileum content of E. Coli, and Enterococcus count in plant treated quails in comparison with the control T0.

Key words: medicinal herbs, body performance, blood traits, ileum histology, body weight.

فعالية بعض النباتات الطبية على أداء الجسم، وحالة الدم، التشريح النسيجي للفائفي و المناعة في السمان الياباني

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المستخلص

هدفت هذه الدراسة لمقارنه تأثير مستخلص النباتات الطبية: زهرة الخطمية أو(هيرو) في شمال العراق(Alcea kurdica)، الزعترThymus bovie، مستخلص أوراق الغار laurus nobilis مع مضادين حيويين Rifaximine و Ranitidine في ماء شرب طائر السمان من خلال تأثيرهم على الأداء الإنتاجي، والحالة الفسلجية لطائر السمان في مرحلتي النمو والتطور لغاية عشرة أسابيع، تم توزيع 90 طائر سمان الى ستة معاملات، كل معاملة احتوت على ثلاث مكررات، بمعدل15 طائر في كل معاملة. أظهر تحليل البيانات زيادة معنوية في زيادة وزن الجسم (237.5 و224.1 و234.9)غم لزهرة Hero و والزعتر وأوراق الغار بالنتائج مقارنة بمعاملة السيطرة (188.3) غم. شرب طائر السمان لمستخلصات النباتات في الماء كان له خصائص دم أفضل وخصائص التقطيع النسيجي (عدد خلايا الكأسية، ارتفاع الزغابة، وعمق خبايا ليبركن)، و ارتفاع تراكيز مصل الدمر من البروتين الكلي والجلوبيولين والأجسام المضادة ضد بعض أمراض فيروس الطيور مقارنة بمعاملة السيطرة. في حين وجد انخفاض ملحوظ في مستوى الألبومين، ومحتوى اللفائفي من E.Coli، وعدد المكورات المعوية في السمان المعالج بالنبات مقارنة مع السيطرة T0.

الكلمات المفتاحية: الأعشاب الطبية، أداء الجسم، صفات الدم، أنسجة اللفائفي، وزن الجسم.

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INTRODUCTION

Poultry meat is a very important source of protein; therefore, in many countries increased capital has been invested in the poultry industry and especially in quails. As a member of the pheasant family, the quail known as the smallest bird. The amount of protein in the meat is 5-10 percent more than other birds and ruminants (15). Quail meat is also a good source of niacin, phosphorus, copper, thiamine, riboflavin, vitamin B6, zinc, iron, and selenium (32). Nowadays, natural diet additives gained more popularity because of increased health issues regarding synthetic nutrients. Therefore, researcher's persuasion for alternatives to replace synthetic sources (antibiotics and drugs) as diet enhancement in poultry farming becomes an important subject (1). Rifaximin and ranitidine were known as gastrointestinal-selective antibiotics with antiulcer and antimicrobial activities (22, 23). But after the ban of nutritive antibiotics in the European Union, interests in plant additives as poultry feed ingredients become risen continuously (3, 16). Medicinal plants have been known as a source of natural antioxidants, such as phenolic acids, flavonoids, and tannins (12, 36). Numerous research works have shown the efficacy of these natural compounds in stopping the onset and/or progression of many human diseases (17). The curative enhancement efficacy of antioxidants from plants may be related to their role in counteracting reactive oxygen species (ROS) (34). Previous research also suggested the need of adding antioxidants to animal's diets to minimize lipid peroxidation and maximize productivity (8). *Alcea kurdica* (Gule Hero) and *Thymus bovie* (Thyme) are medicinal plants with antioxidant and antimicrobial activities, at which both of them have been used traditionally as anti-inflammatory, anthelmintic, expectorant, antispasmodic, antiseptic agents because of flavonoids, polyphenols, and oxygenated Monoterpenoid contents (2, 31). *Laurus Nobilis* (bay leaf) also known as a traditional medicinal plant with antioxidant activity because of phenolic and flavonoid contents at which its essential oil served as antifungal, the antibacterial activity against various gram positives, negative bacteria, and fungus species in the various extracts (10). Another study also reported that bay leaf essential oils have been used to increase appetite and enhance immunity (20). In that regard, on that regard, the present study was set to investigate and compare the effects of Hero flower, Thyme and bay leaf extracts in quail drinking water by emphasizing growth performance, intestinal morphology as well as immune, bacterial and physiological status.

MATERIAIS AND METHODS

Sample collection and powder preparation

The plant samples were collected in a wide geographic range near Shaqlawa-Erbil/Iraq. Identification and authentication of the plants done by Biology Deptarment/ College of Education/ Salahaddin University-Erbil. The plant part: Hero flowers (A. kurdica), Bay leaf (*L. Nobilis*), and Thyme (*T. Bovie*) extracts were prepared as mentioned by Mombeini (25).

Experimental treatments

At age of 1day this study started till 10^{th} weeks, a total of 90 quails was distributed into six treatments, each treatment with 3 replicates and 15 chicks. T0 (control without supplementation). T1 (Rifaximine 200 mg) T2 (Ranitidine 200 mg), T3 (Hero flower extract 100mg), T4 (Thyme extract,100mg), T5 (Bay leaf extract 100mg), each substance was added to a liter of drinking water. All tested groups fed a standard diet contains (2900 ME/kcal, 18.52% crude protein, 4.3% crude fiber, 0.45% methionine, 1.02% lysin), and served ad libitum during the study.

Body performance and histological traits

The birds from each replicate were randomly selected to measuring body weight, and gain, feed intake and Feed Conversion Ratio, water consumption, mortality, and feasibility or economic profit were calculated (30).

Estimation of hematological parameters

At the end of the study10^{th} weeks blood samples were collected from nine birds by cutting the jugular vein of each group using EDTA tubes for counting total Red Blood Cells and White Blood Cells by using Nutt & Herrick solution, blood collected in capillary tubes to estimate Packed Cell Volume, mean corpuscular volume, mean corpuscular hemoglobin concentration Mean corpuscular hemoglobin (MCH), and Drabkin's Reagent is

used for the quantitative, colorimetric determination of hemoglobin (Hb) concentration in whole blood at 540 nm, heterophil and lymphocyte with Wright-Giemsa stains to determine the heterophils and lymphocytes (H/L) ratio (28).

Serum protein and immunology profile

Serum separated from collected blood and determined total protein, globulin, and albumin, according to the instructions of the kits included in the Buyer's Guide for Life Science Bio-compare. Thus, antibody titers against Gamboro, Newcastle, Infectious bronchitis viral diseases were measured by hemagglutination inhibition assay (HI) test (6).

Ileum bacterial count and histological profile: At age 10th week, ileum contents were cautiously kept in sterile petri dishes at -20°C until analyses in the laboratory. One gram of each homogenized sample was collected and transferred into 10 ml sterile saline solution for dilution, then each sample was spread on selective agar plates as follows. De Man, Rogosa and Sharpe (MRS) agar medium was used for lactobacillus bacteria and the Nutrient and McConkey agars were utilized for *E. Coli* (19) and Enterococcus were cultured in semisolid Agar and incubated at 37°C for 24 h for the determining colonies. Intestinal ileum 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 (35) for determining number of goblet cells, villus height, crypt depth and villus to crypt (V/C) ratio (30).

Statistical analysis

All data were analyzed using CRD (Completely Randomized Design) by the SAS institute program (33). Duncan's multiple range tests were used to compare differences among the treatments.

RESULTS AND DISCUSSION

Medicinal plants as diet additives in poultry farming have been used widely due to multiple biological activities that the herbs that play in stimulate the digestive system and enhancing growth rate (18). The results in Table 1 show that adding medicinal plant extract (Hero flower, bay leaf, and thyme) extracts in the drinking water of quail caused significantly ($P \leq 0.05$) higher body weight and body weight gain in T3, T5, and T4 groups. Data analysis also showed significantly ($P \leq 0.01$) higher economic profit of the marketing age of quails' drunk water mixed plant extracts compared with the other treated groups (T0, T1, and T2). As well as feed conversion ratio (FCR) improved in T5, T3, and T4 in comparison with T2, T1, and T0. While nonsignificant differences among all groups in feed intake. Previous researches reported that Hero flower (9), Thyme (21), and Bay leaf (26) extracts possess great antioxidant activity because of their phenolic, flavonoid, and monoterpenoid contents. A study by Abd El-Galil (1) concluding that medicinal plants with characteristics as an anti-inflammatory, restoratives, antibacterial and anti-parasitic will facilitate the performance productivity in poultry. Studies have shown that expansion of the animal's metabolism by herbal plant powder, generally carbohydrates and proteins in the major nutrient would increase growth rates (31). Another cause of weight gaining of quail treated with plant additives may be because of the plant's aromatic content that stimulating the appetite as known that aroma capable of activating the olfactory nerves and gustatory papillae (5). The results of this study accompanied by previous work on the antioxidant efficacy of plants toward increasing egg and body weight as well as improved Feed Conversion Ratio (FCR) in quails (4). No significant changes have been seen in feed intake of all experimented treatments with no mortality during the experimented period Table 1.

Table 1. Effect of some medicinal plants on body performance of Japanese quail at age 10t^{h} wk

Traits	T0	T1	T2	T3	T4	T5	SEM
Initial weight (g)				8.8			
Body weight (g)	197.1^{b}	219.3^{ab}	217.0^{ab}	246.3^{a}	232.9^{a}	243.7^{a}	12.3
Body weight gain (g)	188.3^{b}	210.0^{ab}	208.2^{ab}	237.5^{a}	224.1^{a}	234.9^{a}	9.6
Feed intake (g)	725.0^{a}	732.9^{a}	730.8^{a}	729.1^{a}	714.9^{a}	716.4^{a}	8.9
FCR (g feed/ g BWG)	3.85^{a}	3.49^{ab}	3.51^{ab}	3.07^{b}	3.19^{b}	3.05^{b}	0.38
Economic profit ($)	0.93^{c}	1.35^{b}	1.39^{b}	1.75^{a}	1.54^{ab}	1.70^{a}	0.25

T0: control, T1: Rifaximine 200 mg/L water, T2: Ranitidine 200 mg/L water, T3: Hero flower extract 100 mg/L water, T4: Thyme extract 100 mg/L water, T5: Bay leaf extract 100 mg/L water. $^{a, b, c}$ means within columns with different superscripts differ significantly at (P≤ 0.01) & (P≤ 0.05). The same superscripts among the treatments mean insignificant. FCR: Feed conversion ratio

The results in Table 2. show that erythrocytes profile (total counts of RBCs, Hb concentration, and PCV%) and leukocytes profile (total counts of WBCs and lymphocyte%) were significantly (P≤0.01) increased in the T3 group followed by T4 and T5 experimented treatments. While the lowest erythrocyte and leukocyte profiles were seen in T0, T1, and T2 groups, respectively. The level of MCV, MCH, heterophil%, and H/L ratio were significantly (P≤0.05) lower in the T3, T5, T4, T2, and T1 treated groups, respectively in comparison with the normal control T0. The level of MCHC was significantly (P≤0.05) decreased in the T5, T3, T4 groups in comparison with T1 (Rifaximine 2g/L). Positive feedback of Three experimented plants on the erythrocyte and leukocyte profiles may be linked with their anti-inflammatory and antioxidant activities because of their natural phenolic, flavonoid, and terpenoid contents (9,21, 26). Scientists previously have revealed that adding medicinal plants to poultry drinking water will significantly improve their hematological profile due to the plant's capability in facilitating the erythropoietin process that stimulated kidneys to promote the production of red blood cells and hemoglobin (28). Researchers also have shown the rise of leukocyte production in animals by using medicinal plants and suggest that it could be serve as an immune booster (24). The same results regarding improvement of erythrocyte and leukocyte profiles were found when quails were treated with natural diet additives (27).

Table 2. Effectiveness of some medicinal plants extracts on some hematological parameters of Japanese quail at age 10th wk

Traits		T0	T1	T2	T3	T4	T5	SEM
Erythro-cytes Profile	Total RBCs (10^{6} ml^{-1})	3.209^{c}	4.355^{b}	4.310^{b}	4.990^{a}	4.893^{a}	4.886^{a}	0.329
	Hb (g/dl)	10.59^{c}	12.35^{b}	12.43^{b}	13.75^{a}	13.79^{a}	13.62^{a}	1.07
	PCV (%)	32.79^{c}	37.11^{b}	38.05^{b}	43.29^{a}	43.25^{a}	43.37^{a}	2.16
	MCV (fl)	102.18^{a}	85.21^{b}	88.28^{b}	86.75^{b}	89.49^{b}	88.76^{b}	4.68
	MCH (Pg)	33.00^{a}	28.35^{b}	28.84^{b}	27.56^{b}	28.18^{b}	27.88^{b}	1.55
	MCHC (g/dl)	32.29^{ab}	33.28^{a}	32.67^{ab}	31.76^{b}	31.88^{b}	31.40^{b}	1.37
Leuko-cytes profile	Total WBCs (10^{3} ml^{-1})	22.45^{c}	27.58^{b}	28.10^{b}	32.06^{a}	31.49^{a}	32.35^{a}	1.83
	Heterophil (%)	40.19^{a}	34.92^{b}	33.18^{b}	20.33^{d}	23.86^{c}	21.09^{cd}	1.79
	Lymphocyte (%)	59.81^{c}	65.08^{b}	66.82^{b}	79.67^{a}	76.14^{a}	78.91^{a}	2.49
	H/L ratio	0.672^{a}	0.537^{ab}	0.497^{b}	0.255^{c}	0.313^{c}	0.267^{c}	0.085
Thrombocytes (10^{3} ml^{-1})		32.52^{c}	36.95^{b}	38.83^{b}	45.60^{a}	43.97^{a}	45.33^{a}	2.05

T0: control, T1: Rifaximine 200 mg/L water, T2: Ranitidine 200 mg/L water, T3: Hero flower extract 100 mg/L water, T4: Thyme extract 100 mg/L water, T5: Bay leaf extract 100 mg/L water. $^{a, b, c, d}$ means within columns with different superscripts differ significantly at (P≤ 0.01) & (P≤ 0.05).

Protein profile (total protein and globulin) concentrations in Table 3 were shown significantly (P≤0.01) increased amount total protein and globulin in T3, T5, T4, T1, and T2 respectively compared with control T0. However, albumin concentration was significantly (P≤0.05) decreased in T3, T5, T4, T1, and T2 treatments, respectively compared with control T0. The same Table, also shows the immune status was represented by antibodies titers against (ND, IBD, and IBV) diseases had significantly (P≤0.05) increased

levels in the treatments of medicinal plants extract in drinking water than other treated groups. The highest immune titer was recorded in the T3 group against Newcastle (HI), Gambero disease (IBD), and Infectious Bronchitis Viral (IBV) diseases, respectively. Immune statue was found to be almost the same in T1, T4 and T5 experimented groups. The lowest immune titers showed by normal control T0 group against mentioned viral diseases in comparison with other experimented treatments. Past researches have shown the importance of plant extract as feed additives for appetite stimulator, digestive enzyme secretion, and immune enhancements (18). Regarding our data, studies have shown the antioxidants activity of medicinal herbs can significantly delay or prevent lipid peroxidation as well as decrease protein and carbohydrate denaturation (24).The antioxidant and antimicrobial activities of Hero flower by Bouayed (9), Thyme by Jaradat (21), and Bay leaf by Muñiz-Márquez (26) have been explained previously, at which their phytochemical contents (flavonoids and polyphenols) aid them in scavenging free radicals. Studies also concluded that herbs containing flavonoids, vitamin C, and phenolic compounds will aid in stimulating the immune system by improving lymphocytes, macrophages, and NK cells activities leading to an increase in phagocytosis or interpterion synthesis process (17). The results of this study were similar to the previous study done on the medicinal plant's efficacy on the quail's immunity (27, 37). Similarly, a previous report also showed plant additives caused Physiological and reproductive improvements in Japanese quail (7).

Table 3. Effects of some medicinal plant extract on serum protein profile and immunity by hemagglutination inhibition (HI) test of Japanese quail at age 10th weak.

Traits		T0	T1	T2	T3	T4	T5	SEM
Proteins Profile (g/dl)	Total protein	2.917 ^{c}	4.083 ^{b}	3.982 ^{b}	4.503 ^{a}	4.485 ^{a}	4.488 ^{a}	0.257
	Globulin	1.508 ^{c}	2.912 ^{b}	2.773 ^{b}	3.481 ^{a}	3.340 ^{ab}	3.413 ^{a}	0.205
	Albumin	1.409 ^{a}	1.171 ^{ab}	1.209 ^{ab}	1.022 ^{b}	1.145 ^{ab}	1.075 ^{b}	0.139
Immune statues (Log 2^{n})	Newcastle disease (HI)	4.50 ^{b}	7.50 ^{a}	7.00 ^{a}	8.00 ^{a}	7.50 ^{a}	8.00 ^{a}	0.495
	Gamboro disease (IBD)	3.22 ^{b}	5.00 ^{a}	5.33 ^{a}	5.67 ^{a}	5.33 ^{a}	5.33 ^{a}	0.310
	Infectious Bronchitis Viral (IBV)	2.50 ^{b}	3.67 ^{a}	3.50 ^{a}	4.00 ^{a}	4.00 ^{a}	3.75 ^{a}	0.297

T0: control, T1: Rifaximine 200 mg/L water, T2: Ranitidine 200 mg/L water, T3: Hero flower extract 100 mg/L water, T4: Thyme extract 100 mg/L water, T5: Bay leaf extract 100 mg/L water. $^{a, b, c}$ means within columns with different superscripts differ significantly at (P≤ 0.01) & (P≤ 0.05).

The results of the ileum bacterial profile in a Table 4 show that the number of *Lactobacillus* counts was significantly (P≤0.01) higher in T3, T5, and T4, respectively compared with T1, T2, and T0. However, *E. Coli* and *Enterococcus* counts had significantly (P≤0.01) lower numbers in T3, T4, T5, T2, and T1 compared with the control T0. The results of this study are in accordance with previous findings regarding the antimicrobial activity of Hero flower, which showed great microbial inhibition of aqueous extract of Hero flower against *E. Coli*, *Bacillus*, and some fungal species with no inhibition activity toward Enterobacter aerogenes and Staphylococcus aureus. Previous works showed the presence of the Phenolic and flavonoid compounds in Hero flower (Gallic acids and quercetin) (9), Thyme (Thymol) (21) and Bay leaf (kaempferol and quercetin glycosides) (26) could be the reasons behind their inhibitory effect against the growth of *E. coli* and *Enterococcus* bacteria in the ileum of quail's drunk water mixed medicinal plants. Several studies have reported that phenolic compounds mediate the antimicrobial activities of various plant extracts (6,14, 29). The ileum histological study was present in Table 4. The villi height and crypt depth were increased in the plant extract, and antibiotics treated groups. Of which, quails drunk Hero flower extract in the water had significantly (P≤0.01) higher villus height (865) µm than that (843, 815, 774, 752 and 495) µm for T5, T4, T1, T2, and T0, respectively. The T3 groups also had significantly (P≤0.01) higher crypt depth (91.92) µm and goblet cells (13.17) numbers than that of T5, T4, T1, T2,

and T0, respectively. Quails with no water treatments (T0) showed poor histological traits regarding villus height (495) µm, crypt depth (80.49) µm, V/C (6.15) µm, and goblet cells number (7.03) µm compared with other treated quails that drunk mixed water additives. Different parts of the small intestine are the areas for absorption of various nutrients, and the structure is related to their function. So, changes in feed and feed additives could be affect the morphology of the intestine at different parts. It is well known that improvement of nutrient absorption occurs by increasing the villi height and decreasing crypt depth. Also, increased intestinal villi height and height to crypt depth ratio and decreased crypt depth by plant extracts could be attributed to the decreased renewal rate of intestinal cells as a result of antioxidant properties and antimicrobial actions of plant extracts (29). Therefore, Hero flower, Thyme, and Bay leaf extracts could be increasing the absorption of nutrients by affecting the height and crypt depth of the intestine villi and increasing goblet cell numbers. In accordance with this study, supplementation of different levels of thyme oils in quail's diet due to their thymol content (35%) improved intestinal morphology (11). In contrast, the results of a study on rats showed that the villi width significantly increased in the duodenum and jejunum of the rats fed thyme volatile oil (23). Accordingly, previous reports showed a significantly increased villi height to crypt depth ratio by thymol compared to the basal diet in a state where birds are exposed to pathogens (13).

Table 4. Effect of some medicinal plants extracts on ileum bacterial and histological traits of Japanese quail at age 10th wk

Traits		T0	T1	T2	T3	T4	T5	SEM
Bacterial profile (CFU×105/g)	*E. Coli*	8.02 ^{a}	3.01 ^{b}	3.15 ^{b}	3.11 ^{b}	3.00 ^{b}	3.00 ^{b}	0.428
	Enterococcus	6.83 ^{a}	2.55 ^{b}	2.51 ^{b}	2.55 ^{b}	2.88 ^{b}	2.68 ^{b}	0.503
	Lactobacillus	4.92 ^{c}	12.08 ^{b}	12.08 ^{b}	13.08 ^{a}	12.70 ^{a}	13.00 ^{a}	0.719
Ileum histology	Villus height (µm)	495 ^{c}	774 ^{b}	752 ^{b}	865 ^{a}	815 ^{ab}	843 ^{a}	76.0
	Crypt depth (µm)	80.49 ^{c}	87.26 ^{ab}	84.97 ^{b}	91.92 ^{a}	87.92 ^{ab}	89.87 ^{ab}	2.79
	V/C	6.15 ^{b}	8.87 ^{a}	8.85 ^{a}	9.41 ^{a}	9.27 ^{a}	9.38 ^{a}	0.920
	Goblet cells No.[1]	7.03 ^{c}	11.69 ^{b}	11.66 ^{b}	13.17 ^{a}	12.66 ^{ab}	12.94 ^{a}	1.02

T0: control, T1: Rifaximine 200 mg/L water, T2: Ranitidine 200 mg/L water, T3: Hero flower extract 100 mg/L water, T4: Thyme extract 100 mg/L water, T5: Bay leaf extract 100 mg/L water. V/C: height to crypt depth ratio. $^{a, b, c}$ means within columns with different superscripts differ significantly at (P≤0.01) & (P≤0.05).

CONCLUSION

The current research draws attention to compare three traditional medicinal plant additives in quail's drinking water with two antibiotic drugs and a control group. Their effect caused an increase in the body weight and gain, economic profit (feasibility) of birds by maximizing the absorbed nutrients based on their positive effect on the hematology, immune status, intestinal morphology, and Lactobacillus population in the ileum of quails. *A.kurdica , L.nobilis, and T.bovie* extracts were more influential due to their higher contents of gallic acid, quercetin, and kaempferol glycosides compared with the antibiotic drugs and control groups. Moreover, *A.kurdica* extract could compete with the antibiotics to fight intestinal microbes such E.Coli and Enterococcus. As result, adding plant extracts to quails drinking water could be a better choice for increasing poultry production than antibiotics with fewer adverse effects, which positively reflects on the profitability of birds.

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