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The Use of *Nigella sativa*, *Pimpenella anisum* and *Thymus vulgaris* Mixture in Female Broiler Rations

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to investigate the influence of feeding crushed *Nigella sativa* (NS), *Pimpinella anisum* (PA) and *Thymus vulgaris* (TV) mixture as a natural growth promoter on growth performance, antibodies titer (Abs) and heamatological profiles of vaccinated and unvaccinated female broilers.
Study Design: A total of 400 one- day old Lohman female broiler chicks were weighed and divided into 4 treatments (4 replicates X 30 chicks each).
Place and Duration of the Study: female broiler chicks were raised in an open-sided house at Animal Research Centre at Jordan University of Science and Technology. The experiment lasted for 42- d of age.
Results: The statistical analysis prove that NS, PA and TV mixture significantly increased live body weight (LBW), body weight gain (BWG) and improved feed conversion ratio (FCR). Moreover, medicinal plants mixture increased Abs production against Newcastle (ND), infectious bronchitis (IB) and infectious bursal (IBD) diseases at 21- and 42- d of age. Meanwhile, 2% crushed NS, PA and TV mixture increased total WBC's Monocytes count Heterophils: lymphocytes ratio at 21- and 42- d of age.

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Conclusion and Recommendations: In conclusion, our study clearly reveals that crushed NS, PA and TV mixture improves growth performance, Abs of female broilers. Therefore, it could be used as a natural growth promoter. It has been suggested that further studies be done on each active components of the studied medicinal plants.

Keywords: Antibodies; female broilers; growth performance; hematological profile; medicinal plants mixture.

1. INTRODUCTION

As a result of the significant progress in genetic selection of meat-type chicken, broiler production is more efficient. Therefore, attaining high production levels became very challenging without the use of a number of feed additives. A wide range of additives such as antibiotics [1] and probiotics [2] had been used to improve production. Therefore, fewer of antibiotics growth promoters (AGP) are being used in broiler production. To minimize the loss in growth, there is a need to find alternatives to AGP. In this view medicinal plants and their principal secondary metabolites are used extensively and becoming important due to their wide range of activities. Medicinal plants efficiency on broiler immune system has been reported [3,4]. Moreover, it has been demonstrated that medicinal plants are becoming more important due to their antimicrobial, stimulating effects on birds digestive system through increasing their digestive enzymes and improving utilization of digestive products through enhancing liver function [5,6]. In diseased chicken (either infected with avian *Mycoplasma gallisepticum* or *Eimeria tenella*) it has been demonstrated that plants and their extracts could improve growth performance, reduce populations of coli forms and enhance both cellular and humoral immune response of chickens [7,8].

As an aromatic plant, anise (*Pimpinella anisum L.*) has been used in medications for a long time as a stimulating effect on digestion and as antiparasitic [9], antibacterial [10,11] and antifungal [12]. Furthermore, [13] and [14] reported the beneficial effects of *Thymus vulgaris* in poultry production. Thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isopropyl-2-methyl phenol) are the main phenolic components in *Thymus vulgaris* [15]. However, antibacterial activity of thyme or its main polyphenolic components against several microorganisms such as *Clostredium perferingens*, *Bacillus subtilis*, *E.coli* and *Bacillus cereus* has been reported in previous literature [14,4]. On the other hand, *Nigella sativa* L. was recommended as a non-antibiotics growth promoter for broiler diets [16,17]. These recommendations have undoubtedly made *Nigella sativa* as one of the preferred medicinal plant in poultry production. Moreover, *Nigella sativa* and its oil have been reported to have a broad spectrum of activities against gram-negative microbes [18] and as antiviral agent against murine cytomegals-virus infection [19]. Therefore, this study was designed to examine the effect of using a mixture of *Nigella sativa*, *Pimpinella anisum* and *Thymus vulgaris* as growth promoters on performance, antibody titers, and hematological profiles of vaccinated and unvaccinated female- broiler fed antibiotics-free rations.

2. MATERIALS AND METHODS

2.1 Birds and Rearing Conditions

Four hundred one-day old Lohman female broiler chicks were purchased from a commercial local hatchery and raised in an open sided house at Animal Research Center at Jordan University of Science and Technology. The average weight of chicks was 35.7 g. upon arrival, chicks were randomly distributed into 4 dietary treatments each of 4 replicate (4 treatments x 4 replicates x 25 chicks). Water and feeds were offered ad–libitum. A simple lighting program (23 h L: 1 h D) was used. Ambient temperature was gradually decreased from 33ºC on day one to 25ºC on day 21 and was then kept constant. No antibiotics were used through all the experimental period (42-d). Half of the chicks were vaccinated against Newcastle disease (ND) Hichiner B1 strain at 9-d of age and LaSota strain at 22-d of age, infectious bronchitis (IB) H120 strain at the same age and infectious Bursal disease (IBD) at 13-d of age. The other half of the chicks was not vaccinated against any disease.

2.2 Experimental Rations

Chicks were fed a starter ration from 1 to 21-d of age, and a finisher ration from 22-to 42-d of age

(Table 1). All rations were formulated based on yellow corn and soybean meal to meet the requirement according to the strain guide. Randomized samples from each starter and finisher rations were collected for proximate analysis using the procedure described [20]. Dietary treatments consists of the same starter and finisher antibiotics-free rations (basal) supplemented or not with 2.0% crushed *Nigella sativa* (NS), *Pimpinella anisum* (PA) seeds and *Thymus vulgaris* leaves (TV) mixture (1:1:1) as feed additive through the experimental period.

2.3 Measurements

2.3.1 Live body weight, body weight gain and feed conversion ratio

Immediately after arrival, chicks were weighted and the average weight per chicks were recorded and considered as initial body weight (35.7 g). Live body weight (LBW) and cumulative feed intake (CFI) were measured at 21 and 42-d of age. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated at the same ages.

2.3.2 Antibody titer (Ab ṡ)

At 21- and 42- d of age Ab‘s titer against the most infectious diseases in broiler industry (ND, IB and IBD) were quantified from 5 randomly selected female chickens from each replicate within each treatment. Half of the blood collected in EDTA anticoagulant tubes for lymphocytes, monocytes, red blood cells (RBCs) and haemoglobulin (Hb) determination using the method described by [21]. The second half of blood was received in non-heparinized tubes and centrifuged at 4000 rpm for 15 minutes. Clear serum was used for antibody titer measurements. Antibodies to ND, IB and IBD were detected by enzyme linked immunosorbent assay described by [22] using commercial kits provided by Affini Tech.Ltd., AR.

2.3.3 Statistical analysis

Collected data were statistically analyzed. Pen means were used as experimental units. A completely randomized statistical design was used. Data were subjected to ANOVA using the General Linear Model (GLM) procedure of [23].

Table 1. Composition of experimental ration

Ingredient	Starter %	Finisher %
Yellow corn	60.75	69
Soybean meal (48% CP)	34	26
[1]Broiler concentrate (54% CP)	2	1.75
Limestone	1.25	1.25
Dicalcium phosphate	0.75	0.75
[2]Vitamin: mineral premix	1	1
NaCl	0.25	0.25
Calculated feeding value		
CP (%)	22.62	19.36
ME (MJ/kg diet)	12.46	12.7
EE (%)	2.96	3.17
CF (%)	2.59	2.56
Lysine (%)	1.24	1
Methionin + Cystine (%)	0.73	0.63
Analyzed feeding value		
CP (%)	22.57	19.3
DM (%)	91.95	91.87
EE (%)	2.87	3.1
CF (%)	2.5	2.52

[1] Broiler concentrate provided the following: CP 54%; EE 24%; ME 16.25/Kg diet; Methionine+Cystine 2.05% and Lysine 1.5%.

[2] Vitamin: Mineral premix provided the following: 2,000,000 IU Vitamin A; 400,000 IU Vitamin D3; 400 mg Vitamin E; 200 mg Vitamin B1; 800 mg Vitamin B2; 4,000 mg Nicotinc acid; 2,00 mg pantothenic acid; 300 mg Vitamin K; 200 mg folic acid; 300 mg Vitamin B6; 50 mg Co; 1.600 mg Cu; 6.421 mg Fe; 156 mg I; 12,800 mg Se; 9,000 mg Zn and 100 mg Choline Chloride.

The level of significance was set at P<0.05 or less depending upon the F-values generated. When differences among treatments were found means were separated by the Least Significant Differences (LSD) method following the procedure of [23].

3. RESULTS AND DISCUSSION

3.1 Growth Performance

Table 2 reveals the effect of *Nigella sativa* (NS), *Pimpinella anisum* (PA) and *Thymus vulgaris* mixture on growth performance of vaccinated and unvaccinated female broilers (VFB and UVFB) at 21- and 42-days of age. It can be noticed that, the addition of 2% crushed NS, PA and TV mixture to the antibiotics-free ration (Basal ration) of VFB increased their live BW, BWG and improved FCR compared to other dietary treatments at 21-and 42-days of age. Their P values were 0.019, 0.020 and 0.041 respectively. We hypothesized that the favorable and positive effects of NS, PA and TV mixture could be related to the high nutritive value of such mixture. The previous finding in this regard demonstrated that crushed NS, PA and TV mixture stimulate animal digestive system, particularly proteins, fats and cellulose digestion [24,9,25]. In addition NS seeds have choleric effects producing a definite increase in bile flow [26]. Bile is recognized as emulsifying agent activating the pancreatic lipase that helps in the digestion and absorption of fats and fat soluble vitamins [27]. Many studies so far investigated the chemical composition of *Nigella sativa* seed and reported that the main active components of NS are nigellone, thymoquinone, beta sitosterol, linoleic, archidonic, oleic, myristic, palmatic and stearic acids, vitamins B1, B2, B3 and folic acid plus calcium, iron, zinc, copper and phosphorous [28] which may also explain the favorable effects of NS,PA and TV mixture on growth performance of VFB.

The present findings are in agreement with [29-32]. Nevertheless, health effects of medicinal plants have been associated with its composition of active compounds such as volatile oils, peptides and phenolic compounds [32-35]. It has been reported that *Pimpinella anisum* oil significantly improves daily live BWG and FCR of male broilers [36,37]. However, it also seems that the improvement of PA seeds might be due to the improvement of apparent whole tract and ideal digestibility of the nutrients [6] and increases the effect of pancreatic lipase and amylase [24]. On the other hand, it has been reported that *Thymus vulgaris* has antibacterial activities against a wide range of pathogenic microbial organisms [37]. It has been hypothesized [38] that thymol and carvacrol which are main components of thyme oil bind to the amine and hydroxylamine groups of bacterial membrane proteins and result in the cell death by the permeabilization of the bacterial membrane. Thus it is possible that antibacterial effects of carvacrol and thymol are involved in the effects of thyme on the performance of female broilers. Furthermore, [13] and [14] reported the beneficial effects of thyme in poultry production. Therefore, based on the present results, it could be concluded that 2.0% of crushed NS,PA and TV mixture can be used as a natural growth promoter for broiler production.

3.2 Selected Antibody Titer

The data presented in Table 3 indicated that at 21- and 42- days of age VFM fed the antibiotics-free (basal) ration had the highest Ab‘s titer values against all studied diseases compared to the other dietary treatments. Their P values were 0.021, 0.046 and 0.045 respectively. We assumed that the significant improvement in Ab‘s titer of VFB against ND, IB and IBD diseases might be due to the presence of several active ingredients and high feeding value of such plant mixture. [39] Reported that estragol, anisaldehyde and eugenol are main components of PA, while [15,40] found that carvacrol, anathol and thymol are the main components of TV. Moreover, [41,42] cited that the main active components of NS seeds include carvacrol, thymoquinone, thymol, dithymoquinone and thymohydroquinone which are important pharmacologically active materials. Thymoquinone, representing 18.4-24% of the essential oil NS seeds possesses antibacterial, antioxidant and antiinflamatory activities [43]. Moreover, *Nigella sativa* has been subjected to intensive pharmacological investigations in recent years, due to showing a wide spectrum of activities such as antibacterial and antitumor [44]. On the other hand, [31] reported that replacing bacitracin methylene disalicylate (BMD) by crushed NSS improved significantly Ab‘s of ND and IBD diseases but not IB disease. This improvement is ascribed to NSS oil components such as nigellicine, nigellmine, thymol, carvacrol and thymoquinone. [45,46] Concluded that dietary fatty acids composition can influence immune response in broilers. Moreover, [7,8] demonstrated that plants and their extracts could

improve the growth performance and enhance both cellular and humoral immune responses of diseased chicken (either infected with *Mycoplasma gallisepticum* or *Eimeria tenella*). [47] Demonstrated that a dietary arginine (an essential amino acid) concentration near 1.25% of the diet recommended by [48] should support immune system functions in healthy birds. Essential amino acids play a very important role as a potent immunological modulater, since the cellular metabolic pathway of arginine produces nitric acid [49]. In this regard [50] discovered that nitric oxide production of macrophages is increased by a local concentration of arginine. In a more recent study [51] cited that NS seeds contain proteins including eight or nine essential amino acids of which are arginine, lysine, methionine, tyrosine and leucine. From another point of view, immune system is defined as the system of specialized cells and organs that protect an organism from outside biological influences and antigens, it is also defined as a substance that promotes the generation of antibodies and can cause an immune response [52]. Meanwhile, antibodies known as immunoglobulins, abbreviated Ig and are used by the immune system to neutralize foreign objects, such as bacteria and viruses. We can therefore assure that the active components of NS,PA and TV mixture used in this study may play a role in Ab‘s production as the antigen do, but more research is needed to explain the mechanism through which medicinal plants affect the immunity of broiler chickens.

Our present results are in agreement with previous findings of [53]. [30] indicated that the addition of crushed PA,NS and TV mixture to the basal diet of vaccinated male broilers increased significantly Ab‘s of ND and IB diseases. Moreover, [31] discovered that the replacement of Bacitracin Methylene Disalicylate by anise seed significantly increased Ab‘s against ND, IB and IBD diseases. But the present results disagree with [54] who concluded that the addition of *Thymus vulgaris* extract in drinking water of broiler chickens failed to produce significant differences in Ab‘s against infectious bronchitis at 42- days of age and ND at 21-and 42- days of age.

3.3 Hematological Profile

Results of day 21 (Table 4) and day 42 (Table 5) showed that the addition of crushed NS,PA and TV mixture to the basal diet of VFB increased WBC‘s, heterophils: Lymphocytes ratio and monocytes at 21- and 42- days of age compared with those of other dietary treatments. Their P values were 0.015, 0.042, 0.046 and 0.001 respectively. The increase in total WBC‘s, monocyts counts and hetrophils: lymphocyts ratio of vaccinated female broilers fed the basal ration supplemented with 2% crushed NS,PA and TV mixture noticed at 21- and 42- days of age may be attributed to the active and highly valuable components of these medicinal plants. Previous review of literature concluded that unconventional feed sources affect animal physiology [55]. Furthermore, [26] discovered that essential oils of NS seeds contain crystalline compounds called nigellone or thymoquinone, which has protective effect against diseases. However, [56] reported that hematological constituents reflect the physiological responsiveness of the animal to its external and internal environments including feeds and feeding. Nevertheless, the total count of WBC‘s could be also due to an increase in the membrane protection from auto oxidation. Concerning heterophils: Lymphocytes ratio and monocytes count at 21- and 42- days of age, it can be noticed that VFB fed the supplemented basal ration had significantly the highest values among all treatments. It is well documented that lymphocytes and monocytes perform a specific function against viral infections and several diseases [31,56]. The WBC‘s known to be useful guide to the severity of disease, reflecting the strength of the body’s defense response [57]. The favorable effect obtained in total WBC‘s, monocytes count and heterophils: Lymphocytes might be due to the main components of medicinal plants mixture particularly the essential oils. Furthermore, [58] postulated that crushed NS seeds has an immuno stimulant effect and used it as remedies for diseases, along with other plants extract in traditional medicine. Unfortunately, there is a lack in the literature that can explain how the active components of NS, PA and TV affect blood hematological profile of female broiler chickens. Therefore, there is a serious requirement to start with this field. Our present results are in harmony with those obtained by [59] and [60] who reported that total WBC‘s, lymphocytes and heterophils counts were significantly improved by feeding 0.8% NS seeds compared with control group. The present results disagree with those obtained by [61].

Table 2. Live body weight, body weight gain, cumulative feed intake in (g) and feed conversion ratio of female broilers at 21-d and 42-d of age (Means±SE)

Treatments	21-days of age				42-days of age			
	Live body weight (g)	Body weight gain (g)	Cumulative feed intake (g)	Feed conversion ratio	Live body weight (g)	Body weight gain (g)	Cumulative feed intake (g)	Feed conversion ratio
Basal ration (T1)	541.62±3.69^{d}	505.92±3.60^{d}	850.00±4.37*	1.68±0.05^{a}	1765.34±8.75^{d}	1729.64±5.13^{d}	3371.55±6.45^{b}	1.95±0.03^{a}
Basal ration+ vaccine (T2)	564.15±3.72^{c}	528.45±4.70^{c}	845.60±3.72*	1.60±0.02^{b}	1807.0±9.62^{c}	1771.39±6.65^{c}	3257.35±6.58^{b}	1.84±0.03^{b}
Basal ration +medicinal plants mixture (T3)	582.55±3.70^{b}	546.85±4.72^{b}	846.50±3.67*	1.55±0.04^{c}	1859.64±9.50^{b}	1.823.94±6.87^{b}	3351.45±6.05^{a}	1.84±0.02^{b}
Basal ration + vaccines + medicinal plants mixture (T4)	602.85±3.52^{a}	567.11±5.30^{a}	843.50±3.55*	1.49±0.02^{d}	1996.31±9.65^{a}	1960.61±7.65^{a}	3360.40±6.17^{a}	1.71±0.03^{c}

a-d Means with different superscripts in the same column are significantly different at P<0.05
**not significant*

Table 3. Antibody titer against Newcastle (ND), Infectious bronchitis (IB) and Infectious bursal disease (IBD) of female broilers at 21-d and 42-d of age (Means±SE)

Treatments	21-days of age			42-days of age		
	ND HI	IB ELISA	IBD ELISA	ND HI	IB ELISA	IBD ELISA
Basal ration (T1)	1.29±0.28^{c}	105.71±4.73^{d}	71.86±3.20^{c}	2.86±0.49^{d}	52.7±4.51^{d}	144.86±6.15^{d}
Basal ration+ vaccine (T2)	4.43±0.39^{b}	221.40±6.10^{b}	175.86±5.92^{a}	6.33±1.07^{b}	83.98±6.72^{b}	211.38±8.14^{b}
Basal ration +medicinal plants mixture (T3)	3.85±0.32^{b}	137.05±4.95^{c}	87.15±3.16^{b}	4.30±0.78^{c}	76.64±6.30^{c}	164.70±5.83^{c}
Basal ration + vaccines + medicinal plants mixture (T4)	8.29±0.57^{a}	259.42±7.15^{a}	177.80±6.32^{a}	10.45±1.89^{a}	94.83±6.87^{a}	284.18±8.97^{a}

a-d Means with different superscripts in the same column are significantly different at P<0.05

Table 4. blood hematological profile of female broilers at 21-d of age (Means±SE)

Treatments	WBCs x 10^3/µl	Heterophils: Lymphocytes	Monocytes x10^3/µl l	RBCs x 10^6/µl l	Hb/g/dl
Basal ration (T1)	6.45±0.47^C	2.04±0.17^C	3.05±0.18^C	*2.80±0.20	*12.65±2.46
Basal ration+ vaccine (T2)	10.37±0.90^b	2.69±0.24^b	4.56±0.27^b	2.68±0.17	12.47±2.37
Basal ration +medicinal plants mixture (T3)	9.53±0.87^b	2.60±0.26^b	4.78±0.30^b	2.57±0.18	12.45±2.40
Basal ration + vaccines + medicinal plants mixture (T4)	12.46±0..95^a	3.76±0.28^a	5.50±0.31^a	2.80±0.22	12.50±2.17

a-c Means with different superscripts in the same column are significantly different at P<0.05
**Not significant*

Table 5. Blood hematological profile of female broilers at 42-d of age (Means±SE)

Treatments	WBCs x 10^3/µl	Heterophils: Lymphocytes	Monocytes x10^3/µl l	RBCs x 10^6/µl l	Hb/g/dl
Basal ration (T1)	8.26±0.48^d	1.89±0.05^c	3.25±0.21^c	*2.69±0.20	*12.26±0.67
Basal ration+ vaccine (T2)	11.06±0.78^b	2.04±0.04^b	4.75±0.32^b	2.70±0.25	12.30±0.54
Basal ration +medicinal plants mixture (T3)	9.96±0.60^c	1.87±0.04^c	4.80±0.27^b	2.79±0.22	12.70±0.49
Basal ration + vaccines + medicinal plants mixture (T4)	12.95±0.75^a	2.19±0.06^a	6.20±0.35^a	3.05±0.30	12.96±0.53

a-c Means with different superscripts in the same column are significantly different at P<0.05
** Not significant*

4. CONCLUSIONS AND APPLICATIONS

1. 2% of crushed *Nigella sativa*, *Pimpinella anisum* and *Thymus vulgaris* mixture can replace antibiotics as growth promoter.
2. The same mixture improves significantly growth performance and immunity status of female broilers.
3. It has been suggested that further studies be done on each active components of the studied medicinal plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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