The effect of adding(Rose marinusofficinalis)and(thymus vulgaris)to broilersdiet on immuneresponse and some physiological parameters of broilers

تاثير اضافة اكليل الجبل ونبات الزعتر الى عليقة فروج اللحم وتاثيره على الصفاة المناعية الفسلجية

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Abstract

This experiment was conducted on 200 unsexed Hubbard Flex chicks of one day old for 42 days. Chicks were randomly divided into 5 groups (40 chick pergroup) and each group consists of two replicates (20 chick pergroup). A grinded leaf of Rosemary and Thyme was supplemented to the ration in different combinations and percentages to investigate their effect on some physiological and immunological characters. Group 1 fed with regulate ration without any supplementation as control group. Group 2, 3, 4 and 5 were fed with the supplementation of 0.25% mixture of Rosemary and Thyme, 0.50% mixture of Rosemary and Thyme, 0.25% of Thyme and 0.50% of Rosemary respectively. No significant effect was found for all groups in antibody titer against NDV and internal organ's weight; No significant effect was found for all groups in blood pictures (WBCs, RBCs, Hb and PCV).

Key words: Medicinal plant, Rosemary, Thyme, blood and immunity.

الخلاصة Abstract

أجريت هذه الدراسة في حقل الطيور الداجنة في كلية الطب البيطري – جامعة بغداد لمدة (42) يوما لمعرفة تأثير نباتي أكليل الجبل والجزء المستخدم منه (الأوراق) ، ونبات الزعتر والجزء المستخدم منه (الأوراق) عن طريق إضافتهما إلى العلف بشكل مسحوق طيلة مدة التجربة ، والبالغة 6 أسابيع ودرست تأثير كل منهما على بعض الصفات الدموية لفروج اللحم . استخدمت في هذه التجربة 200 فرخ لحم غير مجنسة نوع (Habbart Flex) بعمر يوم واحد . وزعت 200 فرخ بصورة عشوائية على 5 معاملات (بواقع 40 فرخا لكل معاملة) قسمت كل معاملة إلى مكررين ، وكان تقسيم المعاملات التغذوية كالآتي , المعاملة الأولى : أعطيت عليقة اعتيادية بدون إضافة أي مادة (معاملة السيطرة) .

المعاملة الثانية : أضيف 0.25 % خليط من نباتي أكليل الجبل والزعتر إلى العليقة الاعتيادية طيلة مدة التجربة .المعاملة الثالثة : أضيف 0.50 % خليط من نباتي أكليل الجبل والزعتر إلى العليقة الاعتيادية طيلة مدة التجربة .المعاملة الرابعة : أضيف 0.25 % من نبات الزعتر إلى العليقة الاعتيادية طيلة مدة التجربة .المعاملة الخامسة : أضيف 0.50 % من نبات أكليل الجبل إلى العليقة الاعتيادية طيلة مدة التجربة . وقد بينت النتائج عدم وجود فروق معنوية بين جميع المعاملات ومعاملة السيطرة بالنسبة لفحص الاضداد واوزان الاعضاء الداخلية , وكذلك عدم وجود فروقات معنوية بين جميع المعاملات ومعاملة السيطرة بالنسبة لخلاية الدم الحمر والبيض وحجم الخلايا المضغوطة وهيموكلوبين الدم .

Introduction

A number of feed additives including antibiotics have been widely employed in the poultry industry for several decades. A manipulation of gut function and microbial habitat of domestic animal with feed additives has been recognized as an important tool for improving growth performance and feed efficiency [6].Moreover, herbs contain active substances that can improve digestion and metabolism and possess bacterial and immunostimulant action of animals [18]. The word rosemary is derived from the Latin word “rosemarinus", meaning sea dew. It was also called “antos" by the ancient Greeks, meaning the flower of excellence [10]. Oil of *Rosemarinusofficinalis* can be used as flavor or perfume, possess carminative properties and has a high degree of inhibition against 25 genera of bacteria and fungi [17]. *Thymus vulgaris* is a medicinal herb in the Lamiaceae family, cultivated worldwide for culinary, cosmetic perennial and medical purposes. This species

has special functions such as antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant [1, 11]. Beneficial effects of herbal extracts or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelminthic actions. Isoprene derivatives, flavonoids, glucosinolates and other plant metabolites may affect the physiological and chemical function of the digestive tract. The stabilizing effect on intestinal microflora may be associated with intermediate nutrient metabolism [4, 12, 13]. According to many in vitro studies, it is thought to stimulate macrophage activity and hence the immune system [8, 21]. In recent years, much effort has been made to identify the potential components in Echinacea plant extract that could account for its in vitro immunostimulatoryeffects [5]. In this study, we aimed the use of rosemary and thyme leaves in broiler nutrition as a natural growth promoting, digestive and immune stimulant substance. For this purpose, the different level of rosemary and thyme leaves were added in basal diet and studied to determine the effect on some blood parameters and immune response compared to the control.

Material and methods:

A total of 42 day-old unsexed broiler chicks (Hubbard flex 200) were weighed and based on completely randomized design assigned to 5 treatment groups, Each group was subdivided into two equal sub group with 5 replicate and 20 bird per each replicate. Water and feed were provided for consumption. All the chickens were fed the similar starter (day 1-21 of age) and grower (day 22-42 of age) diets in pellet form (Table 1). But the pellet mixed with *Rosemarinusofficinalis* and *thymus vulgaris*, And fed on the following ration.

1- The first group was fed on basal diet to the end of experiment (control group).
2- The second group was fed on the same ration by adding 0.25% mixture of rosemary and thyme.
3- The third group was fed on the same ration by adding 0.50% mixture rosemary and thyme.
4- The fourth group was fed on same ration by adding 0.25% of thyme.
5- The fifth group was fed on same ration by adding 0.50% of rosemary.

All treatments (diets) were prepared daily. Bronchitis vaccination against Bronchitis virus was done on the 1th day (in drinking water), and vaccination against Newcastle virus happened by drinking water at 1^{st}, 10^{th}, 20^{th} and 30^{th} days of the experimental period, and vaccination against gumboro virus happened by drinking water at 14th days of the experimental periods. At day 21 and 42of age, three birds per pen randomly were selected, weighed and killed bydecapitation to obtain the immune organs relative weights such as spleen and bursa fabricius(percentage of live body weight).Blood samples were collected from the heart directly at 1^{st}, 10^{th} and 20^{th} days in anticoagulant tubes (citrate sodium 3.6% solution) during a forty minute period. After centrifugation (5000 rpm) for 7 min, blood serum was separated and at the consequent Newcastle and Bronchitis disease virus antibody titers were measured by using the ELAISA reader (Ornest American staff, fax 3200).

Blood collection and evaluation:

At the end of four weeks of feeding, one bird from each of the replicates was selected at random, weekly, for bleeding. Blood was collected by wing venipuncture from the right wing. Five millilitres (5 ml) of blood was gently drawn out with the aid of a 5 ml hypodermic syringe. Three millilitres (3 ml) of the blood was put into a labeled blood collection vial containing Ethylene Diamine Tetra-acetic Acid (EDTA) as anticoagulant and the rest of the blood (approximately 2 ml) put into a vial that contained no anticoagulant. The vial with EDTA was gently shaken to facilitate the dissolution of the anticoagulant in order to prevent clotting of the blood. The blood samples which contained no anticoagulants were kept at room temperature for approximately 45 min in order to clot and the serum decanted into clean, labeled tubes. Hemolyzed blood samples were discarded.

Parameters evaluated or calculated included Red Blood Cell Counts (RBC) Packed Cell Volume (PCV), Haemoglobin (Hb) content and total leucocytes (WBC). RBC was determined with a Coulter Electronic Counter (Model ZF by Coulter Electronics Ltd., London). Values were displayed number of red blood cells (x 1012) per liter of blood. PCV was determined through the Wintrobesmicrohaematocrit technique. Some quantity of uncoagulated blood was allowed to flow by capillarity into capillary tubes sealed at one end and centrifuged at approximately 3000 rpm to separate the blood into its cell and non-cell components. The height occupied by the red blood cells was expressed as a percentage of the column of the whole blood. Haemoglobin (Hb) content was determined with a Cecil colorimeter (Model CE 400 by Cecil Instruments, Cambridge) at a wavelength of 625nm after blood had been mixed with Drabkin's solution in a ratio of 1:250 (blood: Drabkin's solution) and expressed in g/dl units. MCV was obtained as 10PCV/RBC femcolitres. MCH was computed 10Hb/RBC pictogram. MCHC was computed 100Hb/PCV (%). WBC was obtained by mixing one part of blood with 399 parts of physiological saline (v/v) and counting with a Neubauerhaemocytometer under a light microscope.

(Table .1) Composition of experimental diets

Ingredient %	Starter (0-21 d)	Grower (21-42 d)
Corn grain	585 Kg	700
Soybean meal	380 Kg	262
Premix + addition	30 Kg	30
Oil	5 Kg	5
salt	5 Kg	5
Total	100 %	100 %
Protein	23.3 %	18.6%
ME (kcal/kg)	2976	3065
E/P	1:128	1:165
Calcium	1.05	1.00
Phosphor	0.51	0.50
Methionine %	1.8	1.8
Lysine %	1.0	1.0
Minerals	1.2	1.2
Antifungal	2.0	2.0
Anticoccidal	1.0	1.0
Vitamins	1.2	1.2

(Table. 2) Vaccination program of broiler chicks during the experimental period.

Age (day)	Vaccine	Rout of vaccination
1	IB and BI	Drinking water
10	Lasota	Drinking water
14	Prise	Drinking water
20	Lasota	Drinking water
30	Lasota	Drinking water

Blood collection and serum separation: Blood was collected from each bird via brachial vein at days; 14, 21, 28 and 35 of age. Sera were separated, labeled and stored at -20C until further analysis.

ELISA procedure: Batches of sera were subjected to serological test. Antibody titers against NDV were measured using ELISA technique described by [19] and NDV antibody test kit (Synbiotics Corporation, San Diego, USA).

Statistical analysis: Analysis of variance using general linear model (GLM) procedure in the PC-SAS® (1988) was used to estimate the variations among the means. Comparison of means in different groups was made by Duncan's multiple-range test [20] P<0.05 was accepted as statistically significant.

Results and discussion:

(Table: 3) The effect of rosemary or thyme and their mixture on antibody titer against NDV

Parameter	Antibody titer against NDV			
Age	1st	9th	19th	29th
Control	433 ± 0.40	575 ± 0.45	2935 ± 0.45	2201 ± 0.89
Mix 0.25%	501 ± 0.59	407 ± 0.71	2519 ± 0.40	1993 ± 0.52
Mix o.50%	417 ± 0.63	489 ± 0.66	3009 ± 0.91	2088 ±0.67
Thyme 0.25%	377 ± 0.50	503 ± 0.60	2707 ± 0.71	2030 ± 0.78
Rosemary 0.50%	462 ± 0.49	499 ± 0.52	2781 ± 0.63	3001 ± 0.99

Values are means ± standard error. Mean values Similar vertically no significant differences at the level of probability ($p < 0.05$).

Table 3 shows the effects of dietary thyme or rosemary and their mixture supplementation on the results of Elisa antibody titer to Newcastle disease vaccine of broiler chickens. The analysis of variance of the obtained data showed nosignificant ($p < 0.05$) variations in Elisa titer at1st,9th, 19thand 29thdays of broiler chickens fed onthe basal diet or supplemented with rosemary and thyme leaves atdifferent levels and these results are similar to thatconcluded by [2] who reported thatthe mean antibody titer against NDV live vaccineresponse showed no evidence that Habek Mint hadstimulated or suppressed the immune system of thebroiler chickens. The lower results of thyme extract on immune system is probably related to the dose of additives, type of plant, method and preparation period and also vaccination program times and stimulator material that used in our study.

(Table: 4) The effect of rosemary or thyme and their mixture on the internal organs weight of broiler at age of 42 days.

Parameter	Thymus Wt %	Spleen Wt%	Bursa Wt%
Control	0.36±0.07	0.16 ± 0.84	0.21±0.01
Mix 0.25%	0.43±0.13	0.15 ± 0.86	0.19±0.02
Mix o.50%	0.48±0.09	0.16 ± 0.84	0.21±0.02
Thyme 0.25%	0.26±0.05	0.14 ± 0.87	0.21±0.01
Rosemary 0.50%	0.27±0.03	0.16 ± 0.86	0.21±0.02

Values are means ± standard error. Mean values Similar vertically no significant differences at the level of probability ($p < 0.05$).

Internal organs characteristics:

There were no significant differences in carcass, thymus, spleen and bursa percentages of chicks fed different concentrations of Rosemary or thyme and their mixture compared to the control group (Table 4). The negative effect of the higher 2% level of Rosemary could be attributed to the presence of high concentration of essential oil and probably a great part of its components are metabolized and then precipitated in the chicken meat. These results are in agreement with those obtained by [7] And also this agree with [22] reported that immune factors such as bursa and spleen relative weight, and also antibody responses to red blood cell and Newcastle disease virus no significantly difference between 0.1% *thymus vulgaris* extract received birds and control group. The diet which content 5% Rosemary led to significant increase in globulin level, but no significant in liver and bursa weight also antibody titer when added 1% Rosemary in broiler diet [9]

(Table: 5) The effect dietary of rosemary or thyme and their mixture supplementation on blood picture (erythrocyte count (RBCs), leucocytes counts (WBCs), hemoglobin (Hb) and packed cell volume (PCV)%) of broiler chickens.

Parameter	WBCs Cell/mm3	RBCs Cell/mm3	Hb %	PCV %
Control	21.75±0.85	2.0±0.09	10.0±0.58	30.25±1.10
Mix 0.25%	21.50±0.50	2.18±0.26	10.0±0.41	27.75±1.65
Mix o.50%	21.75±0.48	2.02±0.13	8.25±0.75	27.25±2.39
Thyme 0.25%	22.0±0.91	1.98±0.22	9.50±0.65	27.25 ± 2.45
Rosemary 0.50%	20.0± 0.96	2.3±0.12	10.0 ± 0.44	29.30 ± 2.01

Values are means ± standard error. Mean values Similar vertically no significant differences at the level of probability ($p < 0.05$).

Blood picture:

The effects of dietary Rosemary or Thyme and their mixture supplementation on some blood picture of broilerchickens in different groups are presented in Table 5.The results regarding RBCs, WBCs count, HB and PCV showed that there were no significant ($p < 0.05$) increase with mixture (0.25 %, 0.50 %) or Thyme and Rosemary (0.25%, 0.50%) respectively when compared with control. Hematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in hematological parameters have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on any living creature. The hematological values obtained in this study indicated no significant impact of Thyme on RBC and WBC counts, hemoglobin content and hematocrit percentage. Reports on the effect of Thyme supplementation on blood hematological parameters are very scarce. Unlike our observation [3] showed that feeding diets were supplemented with oil extract derived from Thyme and cinnamon to broilers, which significantly increased RBC, HCT, Hb and WBC values compared with the control group this results agree with [14] In conclusion, the results suggest that supplementing broilers diet with 5 g/kg Thyme could indicate favorable influences of antibiotic growth promoter effect on performance of broilers without any significant impact on immune responses and blood parameters.these low results of these herbs in blood picture may be due to low of concentration in diet or may not be affected in the chicken when you add in the diet compared added to drinking water after these herbs are extracted. These results are in agreement with those obtained by [16].

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