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Effect of Some Libyan Medicinal Plants on Hematological Profile, Cholesterol Level and Immune Status of Broiler Chicken.

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ABSTRACT

A study was conducted to determine the effect of three native plants from El-Jabal al ELAkhdar, (Libya) on hematological profile, cholesterol and immune response to ND vaccine in broiler chickens. A total of 1260 one-day-old male Cobb chickens were used in the experiment. Chickens were assigned to 7 treatment groups (6 replicates per treatment). The dietary treatments included basal diet with no additives (control), and 6 other dietary treatments (*Arbutus pavarii*, *Salvia officinalis* and *Zizyphus Vulgaris*) each of which was added at the rate of 0.5g and 1g/kg of basal diet. Results explicitly revealed that no significant change in TLC between the treated and control groups has been recorded. A significant heteropenia was recorded at the third week of treatment with 1% *A. Pavarii 0.5 S. Officinalis and 0.5 Z. Vulgaris* when compared to control group. However, the lymphocytic count show a significant increase (P≤0.05) in all treated groups compared to the control groups. Immunologically, an enhanced humeral immune response was very obvious based upon the significant elevation of antibody titer at the third week of treatment for all groups received 1 % of the three types of plants. Interestingly , cholesterol levels were significantly elevated at the six week of treatment with 0.5 % *Z. Vulgaris* while highest decline in cholesterol levels were recorded in group that have received *0.5 S. Officinalis.*

Keywords: *A. Pavarii, S. Officinalis, Z. Vulgaris,* hematology, cholesterol, immunity

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INTRODUCTION

The unique Libyan environment allows a fairly tremendous plant biodiversity. Only few hundreds are used by native apothecaries in conventional medicine. Libya lacks a scientific data about the bioactivity, safety and the health benefits of the local plants. It is worthy investigating the botanical resources of Libya with intention to identify and exploit aspects that provide safe /effective remedies for ailments of both microbial and non-microbial origins. In Libya, around hundred plant species are traditionally used for both medicinal and non-medicinal purposes. The majority of those species grow at Eljabal El-Akhar (Green Mountain) region.

The medicinal plants' mechanistic functions may include improving the physical conditions of gut ecosystem and enhancing function of immune system of chickens [1]. They also cause a remarkable increase in RBC and WBC counts which could be attributed to the direct stimulating effect on the hematopoietic tissue [2] or the production of specific or non-specific antibodies against different antigens [3].

Interestingly, several published literatures have declared that the addition of natural feed additives to poultry diets have no adverse effects on blood components. However, a reduction in total blood cholesterol was reported by Osman [4] . A possible reason could be lipolytic effects of the active substrates of Rosemary, Marjoram and Sweet Basil supplementation [4]. A great majority of natural feed additives such as powdered green tea flower inhibit lipid metabolism by interfering with cholesterol micelles solubilization through the excretion of fecal bile acid cholesterol [5]. It is also reported that green tea like most of natural feed additives reduces pancreatic lipase and gastric lipase activities which in turn result in a drastic decline or even inhibition of gastric digestion of fats [6].

Supplementing broilers diets with different levels of natural feed additives positively improve their immunological status as indicated by increased levels of globulin.

In most of feed additives containing medicinal herbs like black seed or garlic the improvement in immune response is mainly related to the biological function of these herbs [7].According to many *in-vitro* studies, it is thought to stimulate macrophage activity and hence the immune system [8-9]. Recently, rigorous efforts have been made to identify the potential components in Echinacea plant extract that could account for its *in-vitro* immuno-stimulatory effects [10]. In a recent study, Asheg et al. [11] has studied the effect of *Arbutus pavarii*, *Salvia officinalis* and *Zizyphus Vulgaris* on the growth performance and intestinal bacterial count of broiler chicks. They concluded that the three aforementioned medicinal plants have potential immune-modulating effects on the treated chicks.

The current research was conducted to determine the effects of the three Libyan mountains medicinal plants on hematological profile, cholesterol and immune status of broiler chickens

MATERIAL AND METHODS

Chicken and housing

A total of 1260 one day old male Cobb chicks (Cobb Germany) were used in the experiment. Chicks were wing tagged, weighed and placed in floor pens with a wood-shaving floor (30 chicks per pen; size: 2.3x 1.2m). Chicks were assigned to 7 treatment groups (6 replicates per treatment). The dietary treatments included basal diet with no additives (control), and 6 other dietary treatments (*Arbutus pavarii*, *Salvia officinalis* and *Zizyphus Vulgaris*) each of which was added at the rate of 0.5g and 1g/kg of basal diet. Plants were collected from El-Jabal al ELAkhdar, Libya. The whole aerial parts of the plants were air-dried and ground to coarse powder. The diets were manufactured at a commercial company. As described previously Asheg et al. [11]

The feeding program consisted of a pre-starter diet fed from day one to day 14 and a finisher diet fed from day 15 to day 42. Water and feed were available ad libitum and the temperature was gradually decreased from 37° C to 25° C till the end of the trial (42 days). All birds were vaccinated according to the vaccination program implemented by the Department of Animal Health, Libya. The experiment was conducted at the farm of Faculty of Agriculture, University of Tripoli. One chick from each replicate was sacrificed at a weekly interval

for blood samples' collection to monitor the heterophil, lymphocyte,, cholesterol and antibodies' titer against ND vaccine.

Blood constituents and cholesterol examination

Determination of TLC

Blood samples were collected in heparinized vials (0.2 ml of 1% heparin/ 5ml of blood). Total leukocytic count were determined with aid of an improved Neubauer counting chamber using method described by Natt and Herrick's, [12] in which 20 µl of blood was added to 4 ml of Natt and Herricks solution. The leukocyte stained dark blue and total leukocyte concentration was obtained by counting all leukocytes in the nine large squares at the ruled area of the counting chamber using the following equation which described by Campbell and Ellis, [13]:

$$TLC/mm^3 = \{total\ cells\ in\ 9\ squares + 10\%\ of\ total\ cell\} \times 200$$

Determination of DLC

A fresh drop from each blood sample was smeared on clean glass slide and air dried before staining with Wright-Geimsa stain. One hundred white blood cells were counted under oil immersion and results were expressed in percentage.

Cholesterol

Plasma cholesterol was measured using ready to use commercial kit from (Biomaghreb, Morocco), utilizing the method described by Meiattini et al. [14]. Cholesterol esterase (CHE) hydrolyses the esterified cholesterol to free cholesterol, which gets oxidized to form hydrogen peroxide. Hydrogen peroxide further reacts with phenol and 4-aminophenazone, by the catalytic reaction of peroxidase to form a red colored complex of quinoneimine. The intensity of color formed is proportional to the amount of cholesterol concentration in sample and was expressed as mg/dl.

Immune responses

ELISA test

The sera were separated and diluted ten-fold (1:10) with sample diluents prior to examination. The ELISA was carried out to investigate the presence of antibodies against NDV using BioChek antigen Test Kit the procedure steps of the ELISA test was followed according to the manufacturer' recommendations. The absorbances values were evaluated spectrophotometrically by automated microplate ELISA reader (Elx800) from BIO-THEK (UK) Ltd. The absorbance values were measured at 650 nm. The ELISA results are expressed as the ratio between the sample assayed and the (S/P).

Statistical analysis

Statistical analysis system SAS,[15] was used for analysis of variance. Duncan`s multiple range test was used to compare between means Duncan, [16].

RESULTS

Total Leucocytic Count (TLC)

There was no significant change in TLC in all treated groups and control during the first weeks of live. At the second weeks the 1% *Arbutus pavarii* showed a significant increase (P≤0.05) in TLC compared to 0.5 *% Arbutus pavarii, 0.5%* / 1% *Salvia officinalis*. At the third weeks of experiment there was no significant change both between the control and treated groups and among the treated groups. At the fourth week there was a significant increase (P≤0.05) among treated groups with 1 % *Arbutus pavarii* and 0.5% *Zizyphus Vulgaris*

compared to 0.5 % *Arbutus pavarii*, , 0.5% and 1% *Salvia officinalis.* No significant changes were seen at the end of experiment (Table 1).

Heterophils count

There was no significant change in heterophils count through the first and second weeks of experiment. However, at the third week there was a significant decrease (P≤0.05) in 1% *A. Pavarii, 0.5% S. Officinalis and 0.5% Z. Vulgaris* compared to the control group, while there was nosignificant change among the groups and the control during the rest of experiment. (Table 2)

Lymphocyte count

The results showed no significant differences during the first and second weeks of experiments; however a significant increase (P≤0.05) in lymphocyte count in all treated groups compared to the control group was recorded. There were no significant changes during the rest of experiment (table 3).

Blood Cholestrol level

The results showed a significant increase (P≤0.05) in cholesterol level at the sixth week in group treated with 0.5 % *Z. Vulgaris* compared to the control group. The lowest level of cholesterol in blood of chicken was recorded in the group treated with 0.5 % *S. Officinalis* (table 4) .

Table 1: The result of total leukocyte count of chicken (TLC)

Measurement per week	Control	Treatments (g/1kg diet)					
		A. Pavarii		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0. 5	1	0. 5	1
1st w	100.5±.4 a	93.16±5.4a	103.3±22a	94.5 ±5.4a	94.66 ±5.4a	90.16 ±5.4a	102.16±5.4a
2nd w	107.5±8ab	100.1±8b	127.5±8a	93.3±8b	89.5±8b	127.6±8a	107±8ab
3rd w	107.5±8ab	100.1±8b	127.5±8a	93.3±8b	89.5±8b	127.6±8a	107±8ab
4th w	270.5±15.6a	260.3±15.6a	260.6±15.6a	233.3±15.6ab	232.1±15.6ab	260.5±15.6a	225±15.6ab
5th w	235.6±15.3a	261.6±15.3a	213.3±15.3a	246.3±15.3a	256.8±15.3a	261±15.3a	236.5±15.3a
6th w	234.6±17.2a	262.6±17.2	212.3±17.2a	247.3±17.2a	257.8±17.2 a	260±17.2a	237.5±17.2a

ab,c…… = means on the same row have the same letter are not significantly different (P≤0.05

Table 2: The results of heterophils count

Measurement per week	Control	Treatments (g/1kg diet)					
		A. Pavarii		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0. 5	1	0. 5	1
1st w	35.5±2.5a	36.5 ±2.5a	36 ±2.5a	32.83 ±2.51a	37.16 ±2.5a	36.16±2.5a	35.33±2.5a
2nd w	35.5±2.5a	36.5 ±2.5a	36 ±2.5a	32.83 ±2.51a	37.16 ±2.5a	36.16±2.5a	35.33±2.5a
3rd w	32.6±2.2a	26.3±2.2ab	23.6±2.2b	24.3±2.2b	26.5±2.2ab	20.1±2.2b	24.8±2.2b
4th w	18±2.08bc	18±2.0bc	17.3±2.08bc	14.8±2.0bc	21.6±2.08ab	21.8±2.08ab	20.6±2.08abc
5th w	22.1±2.1a	21.3±2.1a	21.6±2.1a	25±2.1a	25.6±2.1a	22.6±2.1a	23.8±2.1a
6th w	29.1±2.5a	24.6±2.5a	27.5±2.5a	26.6±2.5a	24±2.5a	25.8±2.5a	28.1±2.5a

ab,c…… = means on the same row have the same letter are not significantly different (P≤0.05)

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Table 3:**The results of lymphocyte count:**

Measurement per week	Control	Treatments (g/1kg diet)					
		A. Pavarii		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0.5	1	0. 5	1
1st w	59.5 ±2.3a	57.5 ±2.3a	58 ±2.3a	59.8 ±2.3a	57.3±2.3a	56.5 ±2.3a	58.1 ±2.3a
2nd w	59.6±2.3a	68.6±2.3a	71.1±2.3a	69.5±2.3a	67.1±2.3a	73.3±2.3a	67.8±2.3a
3rd w	59.6±2.3b	68.6±2.3a	71.1±2.3a	69.5±2.3a	67.1±2.3a	73.3±2.3a	67.8±2.3a
4th w	75±2.1ab	75.6±2.1ab	76.6±2.1ab	78.3±2.1a	72±2.1ab	70.1±2.1b	75±2.1ab
5th w	70±2a	73.5±2a	73.1±2a	69.3±2a	71±2a	71.5±2a	70±2a
6th w	65.3±2.4a	70.1±2.4a	65.8±2.4a	67.6±2.4a	71.1±2.4a	68.1±2.4a	66.8±2.4a

ab,c…… = means on the same row have the same letter are not significantly different (P≤0.05)

Table 4: Measurement of Cholesterol

Measurement per week	Control	Treatments (g/1kg diet)					
		A. Pavarii		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0.5	1	0. 5	1
6st w	179.40±21bc	232.3±21a b	185.4±21bc	130.5±21c	246.2±21ab	257.4±21a	243.29±23ab

ab,c…… = means on the same row have the same letter are not significantly different (P≤0.05)

Table 5: Results of ELISA test

Measurement per week	Control	Treatments (g/1kg diet)					
		A. Pavarii		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0. 5	1	0. 5	1
1st w	3074.5±647.5bc	4556.2 ±450.4abc	5342.8 ±1842.2 a	3014.5 ±1344.8c	3732.3 ± 2008.7bc	4749.8 ±533.7 a	4669.2 ±661.5ab
2nd w	1550.7±224.2c	2371 ±250b	3821.3 ±862.4a	1635.8 ±156.6c	1433.8 ±225.1c	1784 ±424.2c	2013.5 ±621.4cb
3rd w	1663.3±442 c	1808.3 ±155.90c	2664.5 ±238.9b	1592.5 ±345.2 c	2834.88 ±389.2 b	1478.8 ±279.2 c	5651.2 1349.68a
4th w	4862±1325.2ab	5458 ± 2102.8ab	7241 4720.3a	2969 ±1064.3bc	2658 ±387.5bc	5352 ±387.5ab	6211 ±2117.9ab
5th w	5493±1250.7a	3916 ±2213.9a	6355 ±1787.1a	6777 ±3652.1a	5875 ±1642a	5635 ±2090.5a	6063 ±2199.6a
6th w	4398±1303.2ab	4948.3 ±1819.1a b	3716.6 ±1706.7ab	3246.9 ±1962 ab	2947.2 ±1949.1ab	2881.5 ±921.6ab	2642.8 ±1389.9b

ab,c…… = means on the same row have the same letter are not significantly different (P≤0.05)

Newcastle Disease Virus antibodies' level

A significant increase in antibody titer against Newcastle disease virus (P≤0.05) was recorded in the group treated with 0.5 *A. Pavarii* and 0.5 % *Z. Vulgaris* compared to the control group and groups received 0.5% and *1% S. Officinalis.* At the second week there was a significant increase in antibody titer against Newcastle disease virus (P≤0.05) in group treated with 0.5% and 1% of *A. Pavarii* compared to control group. At the third week, all groups received 1 % of the three used medicinal plants showed a significant increase (P≤0.05) in antibody titer compared to the control groups. Although there was no significant differences among treated groups and control during the rest of experiments , yet, the data showed a significant increase (P≤0.05) in antibody titer in group treated with 1% *A. Pavarii* compared to 0.5 % and 1% *S. Officinalis.*

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DISCUSSION

Our research indicated that the addition of *A. Pavarii, S. Officinalis* and *Z. Vulgaris* to poultry diet had significantly decreased the number of heterophil whereas, a significant increase in lymphocyte count especially, at the third week of life, was seen in all treatment groups compared to control group This result could be attributed to the enhancement of cellular immune response by the natural boosting effects of such medicinal plants. Further, the proven antibacterial effects of these plants against coliform bacteria could have indirectly minimized inflammatory responses [11]. These were manifested by heteropenia and mounted cellular immune response by lymphocytosis. Unfortunately, there is lack of literature on the pathophysiological mechanisms of *A. Pavarii, S. Officinalis* and *Z. Vulgaris* on WBCs differentiation. However, in a moderately recent study, Miroslav et al.[17] have reported a significant increase in the phagocytic activity after the addition of a mixture of *S. officinalis* essential oil together with selenium.

The achieved data indicated that the addition of medicinal plants as feed additive have stimulated the immune response against ND virus which was remarkable in groups received 1% of plants. Osman et al. [4] have reported similar enhanced immune response using different medicinal plants.

The improvement in immune response reported here could be due to enhanced dynamic movement of the heterophils to the intestine which consequentially results in decline of heterophil count in the blood and an increase the count of lymphocytes. A significant increase in lymphocyte count mutually concords with an expected increase in antibodies titer against ND vaccine. Several previous studies explicitly emphasized that medicinal plants like Echinacea purpurea can stimulate macrophage activity and hence the immune system [8].Consistent with results obtained by previous studies, our data revealed a significant increase in lymphocyte count in groups treated with plants additive**.**

The data obtained from our study indicated that the lowest levels of cholesterol was recorded in group received 0.5 % *S. officinali*. This could be due to the interference with enzymes control cholesterol cycle in fat metabolism. Similar studies indicate that the role of phenol compound found in *Rosmarinus officinalis* protect against hypercholesterolemia in rate [17]. On the other side, El-Ghousein and Al-Beitawi [18] and Abdulkarimi et al. [19] postulated hypocholesterolemic and antilipidimic effects of thyme and they related it to the action of thymol and carvacol on reductase enzyme. The rate limiting enzyme of cholesterol is the one who is responsible for reduction of fat absorption from the gut or the lipid catabolism for gluconeogensis**.** Controversially, , our studies indicated a significant increase in cholesterol in group of 0.5% *Z. Vulgaris* but there were no significant changes in other groups compared to the control.

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