British Journal of Poultry Sciences 2 (2): 16-24, 2013
ISSN 1995-901X

DOI: 10.5829/idosi.bjps.2013.2.2.74145

The Effects of Rosemary (*Rosemarinus afficinalis*) and Garlic (*Allium sativum)* Essential Oils on Performance, Hematological, Biochemical and Immunological parameters of Broiler Chickens

[1]Amera S. Abd El-Latif, [2]Nahed S. Saleh, [2]Tamer S. Allam and [2]Emad W. Ghazy

[1]Department of Pharmacology, Faculty of Veterinary Medicine,
Menofiya University, El-Sadat branch, Egypt
[2]Department of Clinical Pathology, Faculty of Veterinary Medicine,
Menofiya University, El-Sadat branch, Egypt

Abstract: This study was conducted to investigate the effects of dietary inclusion of different levels of rosemary and garlic essential oils on performance, hematological, biochemical and immunological parameters of broilers. Seventy five one day-old commercial Ross 208 broiler chicks were assigned to one of five dietary groups (15 birds each). The control group received the basal diet. In addition to the basal diet, the four experimental diets included 100 mg rosemary oil /kg (R_{100}), 200 mg rosemary oil /kg (R_{200}), 100 mg garlic oil /kg (G_{100}) or 200 mg garlic oil /kg (G_{200}). The data of growth performance indicated that supplementation of broiler diets with rosemary oil had no growth-promoting effects. Garlic oil at 100 mg/kg exhibited the best performance. The data of hematological parameters implied a significant increase in packed cell volume in rosemary-treated groupswhile the mean values of hemoglobin demonstrated a significant increase in all treated groups. Significant increase in total leukocytic count was detected in all treated-groups with a marked increase in lymphocytic percent was seen in groups R_{200} and G_{200}. The main changes in blood biochemistry included a significant decreased in serum activity of AST in garlic treated groups. Rosemary and garlic oils supplementation also led to increased serum levels of triglycerides, total cholesterol, low density-lipoprotein (LDL) and high-density lipoprotein (HDL) in broilers. Immunologically, there was a significant increase in phagocytic activity and phagocytic index in all treated groups but all dietary treatments failed to induce any significant effect on antibody titers at any age. Based on the results of this study, it could be concluded that garlic at a level of 100 mg/kg is recommended to be the best dietary treatment throughout improvement of performance of broilers and stimulation of innate immunity by increasing phagocytic capacity of heterophils.

Key words: Broilers · Performance · Rosemary · Garlic · Immunity

INTRODUCTION

Addition of certain antibiotics fed at sub-therapeutic dosages for an extended period of time have been used extensively as growth promoters in livestock feeds for many years and provided economic benefits by increasing growth rate, weight gain and improving feed efficiency of poultry and other livestock [1]. But, recently the use of antibiotics in the poultry industry has been seriously criticized by governmental policy makers and consumers because of the fear that the continuous use of these substances could lead to the development of antibiotic resistant bacteria which are harmful to human health and could be a major contributors to higher feed cost [2].

For this reason, medicinal plants have gained interest in alternative feed strategies for the future [3]. The consumption and demand for these plants and their products including plant extracts or essential oils in poultry rations have been adopted in many countries because of low cost, easy availability and diversified functions in improving growth performance in birds [4].

Corresponding Author: Nahed S. Saleh, Department of Clinical Pathology, Faculty of Veterinary Medicine, Menofiya University, El-Sadat Branch, Egypt. Tel: +201275353696.

Furthermore, plant essential oils have been also proven to control pathogens due to their immunostimulant, antimicrobial and antioxidant activities [5-8].

Rosemary (*Rosemarinus afficinalis*) is an aromatic plant and one of the medicinal plants that is commonly used in the popularity prescription as a spice and a flavoring agent in food processing [9]. The most important constituents of rosemary are carnosal, carnosic acid, caffeic acid and its derivatives such as rosmarinic acid. Rosemary and its constituents were known to have powerful antioxidant activity and thus a therapeutic potential in treating many disease conditions [10, 11].

Garlic(*Allium sativum*) is considered by herbalists to be one of the most essential and useful herbs grown in many areas throughout the world and used for medicinal purposes[12]. The plant has been used for many years to prevent health problems and has been proven to treat various fungal, viral and bacterial infections in addition to the intestinal parasites[13, 14].

The informations about the effects of rosemary and garlic essential oils as growth promoters in certain aspects such as hematologic, biochemical and immunological changes are still not clarified, thus the objective of this study was to investigate the effects of using different levels of rosemary and garlic essential oils on growth performance of broiler chickens with particular emphasis was placed on hematological, biochemical and immunological changes associated with their use in these birds.

MATERIALS AND METHODS

Plant Essential Oils: Essential oils of rosemary and garlic were considered 100% pure. The oils were purchased from a local commercial market (Haraz Company for Medicinal plants), Cairo, Egypt.

Birds: This study was carried out on 75 one day-old commercial Ross 208 broiler chicks obtained from a local company for chicken production (El Sadat City, Menofiya, Egypt). Upon arrival, birds were weighed and kept under good hygienic measures on floors pens covered with wood shavings that were unused when the experiments started and fed a balanced commercial ration till the end of experiment (Table 1). Fresh tap water was supplied *ad libitum*.

Experimental Protocol: Birds were randomly assigned into 5 equal experimental groups of 15 chicks each based on a c ompletely randomized design. The five dietary

Table 1: Composition of the basal experimental ration

Ingredient	Amount
Crude protein%	22.06
Digestible energy Kcal / kg of diet	3079
Calcium%	1.2
Phosphorus%	0.44
Lysine%	0.95
Methionine and cysteine%	0.82

experimental treatments consisted of a control (C) which received the basal diet, basal diet + 100 mg/kg rosemary oil (R_{100}), basal diet + 200 mg/kg rosemary oil (R_{200}), basal diet + 100 mg/kg garlic oil (G_{100}) and basal diet + 200 mg/kg garlic oil (G_{200}). The treatments were added daily to ration till the end of experiment (42 days). All groups were kept under observation and were vaccinated against Newcastle disease (ND) at 8^{th}, 18^{th} and 32^{th} days of age and Infectious Bursal disease (IBD) at 14^{th} and 24^{th} days of age.

Samples: Blood samples were collected at the end of the experiment and were classified into three parts. The first part was collected on disodium ethylene diamine tetracetic acid (EDTA) for hematological assays. The second part was collected on heparin (20 IU/ml) for evaluating the phagocytic activity of heterophils. The third part was placed in plain centrifuge tubes, left to clot then centrifuged for separation of serum. Serum samples were stored at -20°C until assayed for the rest biochemical parameters. Another blood samples were collected at 21, 28, 35 and 42 days of age and serum samples were separated and stored for measuring antibody titer against ND and IBD viruses.

Evaluated Parameters

Growth Performance Parameters: Birds were weighed at first day of age as one day-old weight and then live body weights (LBW) (gm) were recorded at 42 days of age. Feed residues and thus average feed intake (FI) (gm) were recorded daily. The average daily body weight gain (BWG) (gm) and feed conversion rate (FCR) were calculated over this period (42 days). The body weight gain was determined as the difference between the weights of birds at the beginning and end of experiment. Feed conversion rate was determined as FI divided by BWG [15].

Hematological Parameters: Hematological parameters such as packed cell volume (PCV), hemoglobin (Hb), red blood cell count (RBCs) and total white blood cell (WBCs) and differential leukocytic counts were assessed according to the routine hematological procedures for avian [16].

Blood Biochemical Parameters: Serum samples were analyzed for total protein (TP), albumin (Alb), uric acid (UA), serum enzymatic activities of alanine amino transferase (ALT) and aspartate amino transferase (AST), triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). All these parameters were assayed using spectrophotometer and commercial test kits of Randox (Antrim, UK) and following the manufacturer's instructions. Globulin concentration (Glob) in serum was computed by subtracting albumin concentration from total proteinand consequently albumin to globulin ratio (A/G) was calculated.

Immunological Parameters: Phagocytic activity (PA) of polymorphnuclear cells using Candida albicans and phagocytic index (PI) were performed according to the method described by Kawahara *et al*. [17]. Humoral immune response was investigated by detecting serum antibody titers against ND and IBD viruses by hemagglutination inhibition test (HIT) according to the method described by King and Seal [18]. Lymphoid organs (bursa of Fabricius and spleen) were collected from slaughtered chickens at the end of the experiment and their relative weights as two immune indexes were determined.

Statistical Analysis: The data were presented as mean± standard error (SE) and were subjected to statistical analysis using one-way analysis of variance (ANOVA) according to Snedecorand Cochran [19] followed by a multiple comparison Duncan test. Differences at $p \leq 0.05$ were considered significant.

RESULTS

Growth Performance: The data of the effects of dietary supplementation of rosemary and garlic essential oils on performance of broilers shown in Table 2 revealed that there was a significant increase ($P \leq 0.05$) in live body weight and body weight gain in group G_{100} but these values were significantly ($P \leq 0.05$) decreased in group R_{200}. Feed intake increased in groups R_{100}, R_{200} and G_{100}. Significantly ($P \leq 0.05$) higher values of FCR were recorded in rosemary groups while group G_{100} exhibited a significant ($P \leq 0.05$) decrease.

Hematology: With respect to hematological parameters, the data shown in Table 3 clarified that a significant ($P \leq 0.05$) increase in PCV percent was observed in rosemary-treated groups and in Hb values in all treated-groups while red cell count did not show significant changes. Both rosemary and garlic treated-groups

Table 2: The effect of dietary supplementation of rosemary and garlic oils on performance parameters of broilers (Values are means±SE)

	Dietary treatments				
Parameter	C	R_{100}	R_{200}	G_{100}	G_{200}
LBW (gm)	1969.67±6.90^{b}	1962.40±4.4^{b}	1678.27±2.38^{d}	2113.33±16.60^{a}	1946.2±2.54^{bc}
BWG (gm)	45.51±0.55^{b}	45.67±1.15^{b}	38.92±0.68^{c}	49.27±1.96^{a}	45.29±1.87^{b}
FI (gm)	98.89± 3.10^{d}	122.83±5.47^{a}	105.10± 3.11^{b}	101.62± 6.17^{c}	99.73±4.03^{d}
FCR	2.21±0.29^{b}	2.69±0.17^{a}	2.70±0.36^{a}	2.04±0.43^{c}	2.20±0.22^{b}

Means within rows with different superscripts differ at $P \leq 0.05$

Table 3: The effect of dietary supplementation of rosemary and garlic oils on hematological parameters of broilers (Values are means±SE)

	Dietary treatments				
Parameter	C	R_{100}	R_{200}	G_{100}	G_{200}
RBCs (x10^{6}/ µl)	2.54±0.16	2.55±0.11	2.54±0.30	2.48±0.11	2.49± 0.36
PCV (%)	24.60±0.55^{c}	27.40±0.89^{b}	29.60±2.07^{a}	25.60±1.52^{bc}	24.00±1.58^{c}
Hb (g/dl)	7.68±0.15^{b}	10.90±0.47^{a}	12.04±2.04^{a}	10.01±1.16^{a}	11.69±2.99^{a}
WBCs (x10^{3}/ µl)	19.20±1.09^{c}	26.00±2.00^{b}	32.40±1.7^{a}	27.20±2.28^{b}	28.40±1.67^{b}
Heterophil (%)	8.00±0.71	8.80±1.64	8.90±1.41	9.40±2.07	9.50±3.11
Lymphocyte (%)	72.60±0.89^{bc}	73.00±1.22^{bc}	76.40±2.07^{a}	72.20±1.48^{c}	75.00±3.00^{a}
H/L ratio	0.10±0.01	0.12±0.03	0.11±0.02	0.13±0.03	0.13±0.05
Monocyte (%)	17.00±1.00^{a}	15.60±0.55^{a}	12.40±1.81^{b}	16.20±0.84^{a}	12.80±0.45^{b}
Eosinophil (%)	2.40±0.55	2.60±1.14	2.20±0.45	2.00±0.71	2.40±0.89

Means within rows with different superscripts differ at $P \leq 0.05$

Table 4: Serum biochemical parameters of broilers fed rosemary and garlic essential oils (Values are means±SE)

	Dietary treatments				
Parameter	C	R_{100}	R_{200}	G_{100}	G_{200}
T P (gm/dL)	4.12± 0.52	4.39±0.39	4.84±0.88	4.17±0.53	4.46±0.76
Alb (gm/dL)	2.22± 0.54	2.05±0.89	2.03±0.63	1.84±0.58	1.72±0.35
Glob (gm/dL)	1.90± 0.45	2.34±0.32	2.81±0.60	2.32±0.51	2.74± 0.40
A/G	1.62± 0.78	0.97±0.46	1.02±0.55	1.13±0.71	0.67 ±0.20
ALT (U/L)	65.56±2.56	64.11±2.53	65.01±2.82	63.92±2.71	63.81±2.76
AST (U/L)	$378.93±12.07^{ab}$	$356.69±3.67^{bc}$	$362.27±10.88^{abc}$	$333.65±12.76^{d}$	$354.25±2.63^{cd}$
UA (mg/dl)	9.76±0.05	8.62±1.62	9.71±0.88	8.71±0.40	9.22±0.50
TG (mg/dl)	$45.59±2.60^{e}$	$55.89±4.22^{c}$	$61.77±4.56^{b}$	$49.48±3.70^{d}$	$77.98±4.77^{a}$
TC (mg/dl)	$93.36±6.73^{d}$	$125.78±6.69^{a}$	$94.11±6.74^{d}$	$107.35±7.32^{c}$	$120.52±8.75^{b}$
LDL (mg/dl)	$69.69±3.87^{c}$	$94.61±4.19^{b}$	$73.57±4.87^{c}$	$93.16±4.59^{b}$	$102.91±3.91^{a}$
HDL (mg/dl)	$28.68±2.21^{e}$	$69.80±7.85^{a}$	$43.30±4.57^{c}$	$39.51±2.66^{d}$	$61.48±.8.29^{b}$

Means within rows with different superscripts differ at $P \leq 0.05$

Table 5: The effect of dietary supplementation of rosemary and garlic oils on phagocytic activity (PA) and phagocytic index (PI) of broilers (Values are means±SE)

	Dietary treatments				
Parameter	C	R_{100}	R_{200}	G_{100}	G_{200}
PA (%)	$71.60± 4.72^{c}$	$81.00±1.58^{b}$	$89.00± 3.16^{a}$	$80.20±3.96^{b}$	$87.60± 4.56^{a}$
PI	$3.72±0.44^{c}$	$4.39±0.42^{b}$	$5.06±0.30^{a}$	$4.38±0.41^{b}$	$4.61±0.54^{ab}$

Means within rows with different superscripts differ at $P \leq 0.05$

Table 6:The effect of dietary supplementation of rosemary and garlic oils on antibody titers against ND and IBD viruses by HIT (Values are means±SE)

	Dietary treatments				
Day	C	R_{100}	R_{200}	G_{100}	G_{200}
21	2.00±1.00	2.40±0.54	2.60±0.89	2.00±0.70	2.20±0.83
28	3.20±1.30	3.40±0.54	3.80±0.83	3.00±0.70	3.60±1.14
35	4.20±0.83	4.20±0.44	4.60±1.14	4.40±0.54	4.00±1.58
42	4.40±0.54	5.20±1.30	5.60±01.14	4.80±0.83	5.20±1.30

Table 7: The effect of dietary supplementation of rosemary and garlic oils on weight of bursa and spleen of broilers (Values are means±SE)

	Dietary treatments				
Organ	C	R_{100}	R_{200}	G_{100}	G_{200}
Bursa	0.11±0.03	0.11± 0.02	0.13±0.05	0.12±0.03	0.13±0.04
Spleen	0.05±0.01	0.06±0.01	0.07±0.02	0.06±0.02	0.06±0.02

showed a significant ($P \leq 0.05$) increase in total leukocytic count (Table 3). Groups R_{200} and G_{200} exhibited a significant ($P \leq 0.05$) increase in lymphocytic percent and a significant decrease in monocytic percent while no significant differences were observed in heterophilic and eosinophilic percents in all groups (Table 3).

Serum Biochemical Parameters: The effects of the rosemary and garlic essential oils on selected biochemical parameters presented in Table 4 implied that compared to control group, serum levels of TP, Alb, Glob, UA and A/G ratio did not exhibit significant changes. Evaluation of serum enzymatic activities showed a significant ($P \leq 0.05$) decrease in serum activity of AST in garlic-treated-groups while serum ALT activity did not demonstrate significant changes.

In respect to the effects of rosemary and garlic oils on serum lipid profile, the results obtained from Table 4 clarified that serum concentrations of triglycerides and HDL were significantly ($P \leq 0.05$) increased in all treated groups but the increases in total cholesterol and LDL were more pronounced in groups R_{100}, G_{100} and G_{200} (Table 4).

Immunity: Tables 5 and 6 summarize data of the effects of rosemary and garlic essential oils on some immunological parameters. Significant differences ($P \leq 0.05$) from respective control values were detected in phagocytic activity and phagocytic index in both rosemary and garlic groups (Table 5). On the other hand, based on HIT all treated groups elicited no significant differences in antibody titers against ND and IBD viruses at any age

(Table 6). Data of the weights of bursa and spleen summarized in Table 7 showed that, dietary treatment of broilers with different dosages of rosemary and garlic oils did not result in any significant changes in the relative weights of these lymphoid organs in all groups.

DISCUSSION

Withdrawal of antibiotics from poultry foods created need for replacements methods which would influence improvement of health and production traits of broiler chickens. In this respect, considerable attention has been paid to herbal plants as favorable alternatives to antibiotic growth promoters in livestock production to improve the growth feed conversion efficiency and reduce the cost of feed [20]. The main advantage of these compounds over antibiotics is that they do usually not bear any risk regarding bacterial resistance or undesired residues in animal products [21].

Rosemary and garlic are among the plants which in some cases, demonstrated positive effect on health and performance of broiler chickens [10, 11, 22, 23]. But reports about the value of the inclusion of these plants as growth promoters in poultry nutrition are limited and many of researches still under study about the ideal percentage that is used single of it.

In this study, dietary supplementation of different levels of rosemary and garlic essential oils were evaluated for their effects on performance of broilers in addition to other hematological, biochemical and immunological parameters. In respect to performance parameters, the present results showed that supplementation of broiler diets with rosemary essential oil for 42 days had no growth-promoting effects. Similarly, Hernandez *et al*. found no significant differences in body weight gain of broilers when a blend of extracts of sage, thyme and rosemary were added to diets [24]. Additionally, Farhad *et al*. reported that blends of medicinal plants including; garlic, cinnamon, thyme, rosemary and anise induced a significant decrease in the live body weight and daily weight gain at both 21 and 42 days as compared to control birds [25].

Concerning the garlic-treated groups, the data of this study implicated that birds fed garlic essential oil at a level of 100 mg/kg exhibited the best performance which included increased feed intake, increased body gain weight and improved FCR [22, 23, 26]. The possible explanation for this positive growth promoting effect could be attributed to allicin, an organosulfur compound contained in garlic that promotes the performances of the intestinal flora thereby enhance digestion [27]. Additionally, Williams and Losa [1] and Langhout [28] suggested that the improved digestibility of the nutrients leads to a more balanced gut flora with the potential to reduce the proportion of pathogenic bacteria. There is also evidence in the literature that garlic significantly enhances villus and goblet cell numbers in the duodenum, jejunum and ileum of birds and thus activates the entire absorptive process of nutrients [29, 30].

On contrary, Ademola *et al*. [31] concluded that garlic oil supplemented at 100 mg /kg diet in laying hens resulted in increased feed intake and poor feed conversion. Others reported insignificant differences in the feed consumption and feed efficiency in laying hens fed sun-dried garlic [32]. The benefits of the garlic essential oil as growth promoter were not also observed in broiler chickens fedgarlic essential oil at 10, 20 and 40 mg/kg by stomach tube [33]. This contradiction between studies could be possibly due to the difference in form, source and concentration of plant, diets, management and environmental conditions. In the present work, performance decrease with increasing concentration of the rosemary and garlic oils in the respective recipes. This might be attributed to that the higher concentration of the herbs may have an adverse effect on some beneficial microbial populations preventing the plants from exhibiting its positive influence on performance and resulting in a poorer FCR [31, 34].

The data of hematological parameters obtained from this study implied that the addition of rosemary or garlic essential oils to broilers diets did not significantly affect RBC count as they were all similar to those of the control group. Packed cell volume showed a significant increase in rosemary-treated groups while the mean values of Hb content demonstrated a significant increase in all supplemented ones.

The hematological effects of dietary inclusion of herbal plants in broiler and animal rations have been extensively evaluated and were proved in many cases to be positive. Some reported an increase in RBC count for birds fed diets supplemented with garlic extract [35]. Administration of garlic plus ginger also induced a significant increase in Hb content when they added at 10 ml/liter water three days a week [36]. In laying hens, Esonu *et al*. observed a significant increase in Hb level while feeding herbal plant neem to them [37]. These changes could be attributed either to a direct stimulating effect of these herbs on the hematopoietic tissue or to the production of specific or non-specific antibodies against different antigens [38]. On the other

hand, others stated that incorporation of rosemary and garlic into the ration of broilers did not affect the normal haematological integrity of the birds [10, 23].

The findings of the current study in respect to total and differential leukocytic count showed that significant increase in total leukocytic count was detected in all treated-groups with the highest values were recorded in group R_{200} followed by group G_{200} probably due to the significant lymphocytosis seen in the two groups. These results are consistent with some studies which reported that dietary garlic oil at 200 mg/kg induced a significant increase in WBCs count in laying hens [31]. Although, almost no available literatures to explain the effect of rosemary and garlic essential oils on white blood cell differentiation, these effects could be explained by the stimulatory effects of these oils on immune functions and improved immuno-competence of the birds [39].

Evaluation of serum biochemical parameters inthis investigation revealed that dietary supplementation of rosemary and garlic essential oils resulted in asignificant decrease in serum activity of AST in garlic treated-groups while serum levels of totalprotein, globulin, albumin,uric acidand serum ALT activity did not show significant changes.Although we do not have explanation for the decrease in AST activity in garlic treated-groups, these results indicate at least that rosemary and garlic essential oils did not have any deleterious effects on liver and kidney functions.These results are supported by some previous reports which implemented that feeding broiler rosemary at levels of 0.5 and 1gm/kg did not significantly affect total protein, albumin, globulin**,** creatinine and AST and ALT activities [10]. Also this assumption is in line with the conclusion of some researchers who stated that administration of garlic and ginger essential oils at 10, 20 and 40 mg/kg did not induce significant effects in the activities of serum AST and ALT or in blood creatinine level [33]. Furthermore, some herbal plants have been shown to be hepatoprotective and capable of inducing an endogenous interferon [40].

The effects of rosemary and garlic essential oils on lipid profile clarified that with the exception of group R_{200} for TC and LDL, all treated groups exhibited a significant increase in serum concentrations of TG, TC, LDL and HDL. Conversely, some authors observed that dietary rosemary did not significantly affect cholesterol level in broilers [10]. Further, feeding broilers dried garlic powder reduced serum cholesterol levels [23]. The effects of herbal plants on blood lipid profile have been shown to be controversial. Hyperlipidemic effects were seen with some plants [34, 41] and hypolipidemia was reported with others [42]. The discrepancies between studies might be attributed to the differences in herbal plant used, doses, form of plant, route of administration as well as experimental conditions.

A large number of medicinal plants have been claimed to possess immunomodulatory activities and were used to provide alternative potential to conventional chemotherapy for a variety of diseases, especially in relation to host defense mechanism [43]. Immune responses can be classified either as reactions of innate immunity (nonspecific) and reactions of adaptive immunity (specific) mediated by T and B lymphocytes [44]. The most important cells involved in innate immunity include, natural killer cells and the phagocytic cells including macrophages and neutrophils. Phagocytes are known to play an important role in resistance to infection because of their ability to engulf and degrade invading microorganisms and kill them intracellularly [44].

The current findings of the effects of different levels of rosemary and garlic essential oils on innate immune response implied a significant increase in phagocytic activity and phagocytic index in all treated groups with the highest values were observed in R_{200} and G_{200} groups. Similar effects were reported with other medicinal plants. Soltan *et al*. found that dietary anise seeds supplementation at different concentration increase phagocytic activity, index and lymphocytes in broilers [45]. Awaad *et al*. observedthat essential oils of peppermint and eucalyptus significantly increased phagocytic activity of macrophages in chickens [46].

The components of the herbal plants which are responsible for the immune stimulation are not well known. Guo suggested that the mechanism of medicinal plants components may include improving the physical conditions of gut ecosystem and enhancing function of immune system of chicks [47]. The antioxidant nature of medicinal plants also has been suggested to alleviate the negative influence of environmental stressors and improve immune function to combat different types of diseases [7, 10, 11]. Others identified a carbohydrate in the garlic extract that appeared to be responsible for the antitumor immunity [48].

Concerning the effect of rosemary and garlic oils on humoral immune response, the analysis of variance of the data of HIT showed that all dietary treatments used in this study failed to induce any significant effect on antibody titers against ND and IBD viruses at any age. Contradictory findings have been reported by some authors who declared that blends of medicinal plants including; garlic, cinnamon, thyme, rosemary and anise

increased antibody titer against ND at day 33 [25]. However, these findings can be supported by the work of Waihenya *et al*. who reported no change in antibody titers against ND in chickens fed crude extract of aloe secundiflora [49].

Data of relative weights of bursa and spleen in this study showed that dietary supplementation of rosemary and garlic essential oils did not significantly affect the relative weights of these organs. In accordance with these results, Dieumou *et al*. recorded that all organs expressed as a proportion of body weight of birds were not affected by treatment with garlic essential oil at 10, 20 and 40mg/kg [33]. On contrary, others found that relative weights of bursa and spleen were significantly heavier when rosemary was supplied at a level of 1gm/ kg than those of control. However the author in the last study used much larger dose of the rosemary oil [10].

Based on the results obtained from this study it could be concluded that supplementing broilers diet with rosemary oil at levels of 100 and 200 mg/kg and garlic oil at a level of 200 mg/kg did not improve performance of broilers but garlic oil at a level of 100 mg /kg exhibited the best performance parameters. Both oils were not associated with any adverse impacts on hematological and biochemical parameters however they resulted in hyperlipidemic effects indicated by hypertriglyceridemic and hypercholesterolemic effects. Immunologically the two herbs appeared to have immunomodulatory effects particularly on innate immune response indicated by the significant increase in phagocytic activity and index of heterophils but both of them at the tested doses had no effect on humoral immune response and antibody production.

Accordingly, among the dietary treatments used in this study, garlic oil at a level of 100 mg/kg is recommended to be the best treatment throughout improvement of performance of broilers and stimulation of innate immunity by increasing phagocytic capacity of heterophils.

REFERENCES

1. Williams, P. and R. Losa, 2001. The use of essential oils and their compounds in poultry nutrition. World Poultry, 17(4): 14-15.
2. McCartney, E., 2002. The natural empire strikes back. Poult. Int., 41(1): 36-42.
3. Varel, V.H., 2002. Livestock manure odor abatement with plant-derived oils and nitrogen conservation with urease inhibitors: A review. J. Anim. Sci., 80(2): E1-E7.
4. Lewis, M.R., S.P. Rose, A.M. Mackenzie and L.A. Tucker, 2003. Effect of dietary inclusion of plant extract on the growth performance of male broiler chicken. Journal of British Poultry Science, 20: 78-82.
5. Dorman, H.J.D. and S.G. Deans, 2000. Antimicrobial agent from plants: antimicrobial activity of plant volatile oils. J. Appl. Microbiol., 88: 308-316.
6. Azaz, D., F. Demirci, F. Satıl, M. Kürkçüoğlu and K.H.C. Baþer, 2002. Antimicrobial activity of some Satureja oils. Z. Naturforsch., 57c: 817-821.
7. Botsoglou, N.A., P. Florou-Paneri, E. Christaki, D.J. Fletouris and A.B. Spais, 2002. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipidoxidation of breast, thigh and abdominal fat tissues. British Poultry Science, 43(2): 223-230.
8. Thomson, M., K.K. Al-Qattan, S.S. Al-Sawan, M.A. Alnaqeeb, I. Khan and M. Ali, 2002. The use of ginger (Zingiber officinale Rosc.) as a potential anti-inflammatory and antithrombotic agent. Journal of Prostaglandins Leukotriens and Essential Fatty Acids, 67: 475-478.
9. Leung, A., 1980. Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics. New York: John Wiley & Sons, pp: 231.
10. Mona, O., H.M. Yakout, H.F. Motawe and W.F. Ezz El-Arab, 2010. Productive, physiological, immunological and economical effects of supplementing natural feed additives to broiler diets. Egypt. Poult. Sci., 30: 25-53.
11. Al-Kassie, G.A.M., R.A. Abd-Al-Jaleel and A.M. Mohseen, 2011. The effect of a mixture of anise and rosemary on broiler performance. Agric. Biol. J. N. Am., 2(9): 1279-1282.
12. Sankaranarayanan, A., T. Narender, S. Kumar and M. Dikshit, 2007. *Allium sativum* constituents: effect on free radical generation from ratneutrophils. J. Cell Mol. Biol. (Noisy-le-grand), 53: 63-67.
13. Zenner, L., M.P. Callait, C. Granier and C. Chauve, 2003. *In vitro* effect of essential oils from *Cinnamomumaromaticum*, Citrus limon and *Alliumsativum* on two intestinal flagellates of poultry, *Tetratrichomonasgallinarum*and *Histomona smeleagridis*. Journal of Parasitology, 10: 153-157.
14. Okpuzor, J., G. Kareem and C. Ejikeme, 2009. Lipid Lowering Activity of Globimetulabraunii. Res. J. Med. Plant, 3: 45-51.
15. Brody, S., 1968. Bioenergetics and growth. Hafner Publ. Comp: N.Y.

16. Feldman, B.F., J.G. Zinkl and N.C. Jain, 2000. Schalms Veterinary Haematolog. 5th Ed. Philadelphia, Williams and Wilkins, pp: 21-100.
17. Kawahara, E., T. Ueda and S. Nomura, 1991. *In vitro* phagocytic activity of white spotted shark cells afterinjection with Aeromoas salmonicida extraceularproducts. Gyobyo Kenkyu, Japan, 26: 213-214.
18. King, D.J. and B.S. Seal, 1998. Biological and molecular characterization of Newcastle disease virus (NDV) field isolates with comparisons to reference NDV strains and pathogenicity after chickens or embryo passage of selected isolates. Avian Diseases, 42: 507- 516.
19. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. Iowa State, University Press, Ames, USA.
20. Zakeri, A.and P. Kashefi, 2011. The Comparative Effects of Five Growth Promoters on Broiler Chickens, Humoral Immunity and Performance. Journal of Animal and Veterinary Advances, 10(9): 1097-1101.
21. Perić, L., D. Žikić and M. Lukić, 2009. Application of alternative growth promoters in broiler production. Biotechnology in Animal Husbandry, 25(5-6): 387-397.
22. Ademola, S.G., 2004. Growth, hematological and biochemical studies on garlic and ginger-fed broiler chicken. Moor J. Agric. Res., 5: 122-128.
23. Onyimonyi, A.E., P.C. Chukwuma and C. Igbokwe, 2012. Growth and hypocholesterolemic properties of dry garlic powder (*Allium sativum)* on broilers. Afr. J. Biotechnol., 11(11): 2666-2671.
24. Hernandez, F., J. Madrid, V. Garcia, J. Orengo and M.D. Megia, 2004. Influence of two plant extracts on broiler performance, digestibilityand digestive organ size. Poult. Sci., 83: 169-174.
25. Farhad, K., S. Ghorbanali, K. Ahmad and V. Asaad, 2011. Evaluation of different medicinal plants blends in diets for broiler chickens. J. Med. Plant. Res., 5(10): 1971-1977.
26. Suriya, R., I. Zulkifli and A.R. Alimon, 2012. The effect of dietary inclusion of herbs as growth promotes in broiler chickens. J. Anim. Vet. Adv., 11(3): 346-350.
27. Pourali, M., S.A. Mirghelenj and H. Kermanshahi, 2010. Effects of garlic powder on productive performance and immune response of broiler chickens challenged with Newcastle Disease Virus. Global Vet., 4: 616- 616.
28. Langhout, P., 2000. New additives for broiler chickens. World Poultry, 16(3): 22-27.
29. Tatara, R.M., E. Sliwa, K. Dude, J. Mosiewicz and T. Studzinski, 2005. Effect of aged garlic extract and allicin administration to sows during pregnancy and lactation on body weight gain and gastrointestinal tract development of piglets. Bull. Vet. Inst. Pulawy, 49: 349-355.
30. Masoud, A., 2006. Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. J. Poult. Sci., 43(4): 378-383.
31. Ademola, S.G., A.B. Sikiru, O. Akinwumi, O.F. Olaniyi and O.O. Egbewande, 2011. Performance, Yolk lipid, Egg Organoleptic properties and Haematological Parameters of Laying Hens Fed Cholestyramine and Garlic Oil. Global Veterinaria, 6(6): 542-546.
32. Chowdhury, S.R., S.D. Chowdhury and T.K. Smith, 2002. Effect of dietary garlic on cholesterol metabolism in laying hen. J. Poult. Sci., 7(2): 122-128.
33. Dieumou, F.E., A. Teguia, J.R. Kuiate, J.D. Tamokou, N.B. Fonge and M.C. Dongmo, 2009. Effects of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) essential oils on growth performance and gut microbial population of broiler chickensLivestock Research for Rural Development, 21(8).
34. Majid, T., T. Mohsen, A.G. Abas and A.T. Sayed, 2010. Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afr. J. Biotechnol., 9(40): 6819-6825.
35. Elnagar, S.A., A. El-Sebai and M. Abaza, 2003. Efficacy of (Allii) Against fattening in peckin ducks. In the Proceedings of the 2003 World Waterfowl Conference, pp: 7-9.
36. Rehman, S.U., F.R. Durrani, C. Naila, U.K. Rifat and F. Rehman, 2011. Comparative efficacy of different schedules of administration of medicinal plants infusion on hematology and serum biochemistry of broiler chicks. Roavs, 1(1): 8-14.
37. Esonu, B.O., M.N. Opara, I.C. Okoli, H.O. Obikaonu, C. Udedibie and O.O.M. Iheshiulor, 2006. Physiological Response of Laying Birds to Neem (AzadirachtaIndica) Leaf Meal-Based Diets: Body Weight Organ Characteristics AndHaematology. Journal of OJHAS, 5: 972-997.
38. Khodary, R.M., M.H. El-Azzawy and I.R. Hamdy, 1996. Effect of nigella sativa on egg production, hatchability percentage and some biochemical values in laying hens with reference to fertility in cockerels. In the Proceedings of the 1996 Scientific Conference of Assuit University, Egypt, pp: 91-106.

39. Surniyoshi, H., 1997. New pharmacological activity of garlic and its constituents (review). Folia Pharmacologica. Japonica, 110(1): 93-97.
40. Thyagarajan, S., S. Jayaram, V. Gopalakrishnan, R. Hari, P. Jeyakumar, M. Sripathi, 2002. Herbal medicines for liver diseases in India. Journal of Gastroentrology and Hepatitis, 17: 370-376.
41. Bölükbaşi, Ş.C., M.K. Erhan and A. Özkan, 2006. Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers.South African Journal of Animal Science, 36(3): 189-196.
42. Lee, K.W., H. Everts, H.J. Kappert, H. Wouterse, M. Frehner and A.C. Beynen, 2004. Cinnamaldehyde, but not thymol, counteracts the carboxymethyl cellulose-induced growth depression in female broiler chickens. Int. J. Poult. Sci., 3(9): 608-612.
43. Kumar, S.V., S.P. Kumar, D. Rupesh and K. Nitin, 2011. Immunomodulatory effects of some traditional medicinal plants J. Chem. Pharm. Res., 3(1): 675-684.
44. Stafford, J.L., N.F. Neumann and M. Belosevic, 2002. Macrophage-mediated innate host defense against protozoan parasites. Crit. Rev. Microbiol., 28: 187-248.
45. Soltan, M.A., R.S. Shewita and M.I. El-Katcha, 2008. Effect of Dietary Anise Seeds Supplementation on Growth Performance, Immune Response, Carcass Traits and Some Blood Parameters of Broiler Chickens. Int. J. Poult. Sci., 7(11): 1078-1088.
46. Awaad, M.H.H., G.A. Abdel-Alim, K.S.S. Sayed, K.A. Ahmed, A.A. Nada, A.S.Z. Metwalli and A.N. Alkhalaf, 2010. Immunostimulant Effects of Essential Oils of Peppermint and Eucalyptus in Chickens. Pak. Vet. J., 30(2): 61-66.
47. Guo, F.C., 2003. Mushroom and herb polysaccharides as alternative for antimicrobial growth promoters on poultry, M.S. thesis, Wageningen Univ., Netherlands.
48. Corzo-Martinez, M., N. Corzo and N. Villamiel, 2007. Biological properties of onions and garlic. Trends in Food Science and Technology, 18: 609-625.
49. Waihenya, R.K., M.M.A. Mtambo and G. Nkwengulila, 2002. Evaluation of the efficacy of the crude extract of Aloe secundiflora in chickens experimentally infected with Newcastle disease virus. J. Ethno Pharm., 79: 299-304.