



## INFLUENCE OF ADDING GARLIC AND THYME AND THEIR COMBINATION ON IMMUNE RESPONSE AND SOME BLOOD PARAMETERS IN BROILER

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### ABSTRACT

The World Health Organization estimated that 80% of the earth's inhabitants rely on tradition medicine for their primary health care needs and most of this therapy involves the use of plants (especially herbs). Plant and spices as single compounds or as mixed preparations can play a role in supporting both performance and health status of the animal. The aim of the present study is evaluate of the potential of supplementing broiler's diets with garlic (powder) and Thyme (powder) and mixture of garlic and thyme as feed additive on the immune response against NDV and some blood traits of broilers. 200 day-old broiler chicks (Ross 308) were divided into four treated groups (50 chicks per group) with two replicate per each group (25 chicks per replicate). Dietary treatment included (T1) basal diet only (control group), (T2) fed basal diet with 1% thyme, (T3) fed basal diet with 1% garlic, and (T4) fed basal diet with mixture 1% thyme and 1% garlic. The experiment was extended to 35 days. Traits included in this study were ELISA antibody titer against ND virus of 15<sup>th</sup>, and 30<sup>th</sup> day old chicks, RBCs, WBCs, PCV, and Hb. Results revealed that that ELISA antibody titers, total WBCs, total RBCs, PCV, and Hb were increased significantly (p 0.05) in T4 (chicks fed basal diet supplemented with 1% thyme and 1% garlic) and T3 (chicks fed basal diet supplemented with 1% garlic) then T2 (chicks fed basal diet supplemented with 1% thyme) respectively as compared with control group at 15<sup>th</sup> and 30<sup>th</sup> day-old chicks. In conclusion, the results confirmed that supplementing broilers diet with mixture of 1% thyme plus 1% garlic significantly enhanced immune response and blood profile of broiler. These results could be due to interaction between dietary inclusion and synergistic effect of two plants thyme and garlic.

**KEYWORDS:** Thyme, Garlic, Immune system, Blood parameters, Performance, Broilers, Feed additives.

### 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 (Collington *et al.*, 1990). Volatile oil from thyme (*Thymus vulgaris*) thymol and carvacrol, a major component isolated from essential oil of the thyme was assessed for antibacterial and antiviral activity as inhibitors of microbial growth (Dorman and Deans, 2000; Najafi and Torki, 2010). Garlic (*Allium sativum*) is a medicinal herb for prevention and treatment of many diseases (Adibmoradi *et al.*, 2006). It has an antibiotic productive performance when added as a food supplementary in broiler diets, stimulate the immune system, causes quantitative changes of blood leukocytes, enhances digestion, used as growth promoter, increasing body gain, feed intake and feed efficiency (Onibi *et al.*, 2009). Uses of antimicrobial compounds produced by microorganisms have been used in animal rations as growth promoters for many years (Church and Pond, 1988; Barragry and Powers, 1994). Antibiotics affect birds' gut microflora (Botsoglou *et al.*, 2002) and they have been used widely to prevent infections and poultry diseases for the improvement of meat and egg production. However, use of antibiotics is restricted due to drug

resistance in bacteria, drug residue in carcass and also alteration of natural gut microflora (CAFA, 1997). Thus, use of antibiotics growth promoters is restricted in many countries around the world (Thakar *et al.*, 2004). Removing these kinds of growth promoters from broilers' diet result in low growth performance, and less resistance against diseases. Therefore, using other alternatives is being concerned. Several compounds like, enzymes, organic acids, probiotics, prebiotics and phytogetic are used to improve the performance (Patterson and Barkholder, 2003). Recently, aromatic plants, and their associated essential oils or extracts are being concerned as potentially growth promoters. Garlic (*Allium sativum*) has been used as a spice and a native medicine since long ago (Rivlin, 2001). Bioactive components of garlic like sulfur containing compounds (Alliin, Diallylsulfides and Allicin) may be responsible for some specific characteristics of this plant (Amagase, 2001). It has been indicated that these compounds have antibacterial, antifungal, antiparasite, antiviral, antioxidant, antithrombotic, ant cancerous and vasodilator characteristics. Garlic powder as a natural growth promoter can be a potential alternative for common artificial growth promoters like antibiotics and in this respect, it can improve performance and carcass characteristics in broiler chickens (Demir *et al.*, 2003; Lewis *et al.*, 2003). Plant extracts and spices as single compounds or as mixed preparations can play a role in

supporting both performance and health status of the animal (Janssen, 1989; Horton *et al.*, 1991; Bakhtiet and Adam, 1995; Skrabka Blotnicka *et al.*, 1997; Gill, 2000; Manzanilla *et al.*, 2001). 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. The objective of this study was to compare the effects of two commercial plant powders as alternative to antibiotic on immune system, and some blood factors in broiler chickens.

## MATERIALS & METHODS

### Experimental design

Two hundred day-old unsexed broilers chicks (Ross-308) were bought from a commercial hatchery and were divided randomly and equally into four treated groups (50 chicks per group) with two replicate per each group (25 chicks per replicate). Dietary treatment included (T1)

basal diet only (control group), (T2) fed basal diet with 1% thyme, (T3) fed basal diet with 1% garlic, and (T4) fed basal diet with mixture 1% thyme and 1% garlic. An experiment carried out for 35 days. Traits involved in this study were antibody titer against ND virus of 15<sup>th</sup>, 23<sup>th</sup> and 32<sup>th</sup> day old chicks, RBCs, WBCs, PCV, and Hb.

### Rearing program

The chicks were management according to (Aviagen, 2009). Feed and water were provided *ad libitum*. Two types of diets (starter and finisher) were used over the period of experiment (35 days) (Tables 1). Chicks were vaccinated against Newcastle disease (ND) (B1 strain) and infectious bronchitis at the first day of age by spray. While, all others vaccines were administrated with drinking water which includes ND (Lasota strain) at age 10 days, Gumboro (IBD2) at age 14 days, ND (Lasota strain) at age 20 days and ND (Lasota strain) at age 30 days. Vitamin C was added at the rate of 1gm/liter for 3 days after each vaccination and for duration of 3 days.

**TABLE 1:** Composition of experimental diets (starter and finisher) according to (NRC)

Ingredient %	Starter diet	Finisher diet
Yellow corn	36	44
Soybean meal (48% protein)	30	26
Wheat	26	20
Protein concentrate	5	5
Sunflower oil	1.5	3.5
Premix	0.1	0.1
Lime stone	1	1
Salt	0.3	0.3
Dicalcium phosphate	0.1	0.1
Total	100	100
Calculated chemical analysis		
Metabolize energy (kcal/kg)	2926	3097.8
Crude protein (%)	22.4	20.5
Calcium (%)	0.82	0.80
Available phosphorus (%)	0.61	0.58
Methionine (%)	0.61	0.58
Lysine (%)	1.74	1.63

\* Premix produced in Jordan (VAPCO®) which contains: vit A 8000000 IU; vit D3 1500000 IU; vit E 1000 IU; vit K3 2000 mg; vit B1 500 mg; vit B2 500 mg; vit B6 200 mg; vit B12 8 mg; ca pantothenate 400 mg; nicotinamide 6000 mg; folic acid 50 mg; methionine 13 mg; lysine 61 mg; aspartic acid 92 mg; glutamic acid 166 mg; cysteine 1 mg; valine 40 mg; tyrosine 9 mg; glycine 382 mg; arginine 117 mg; leucine 48 mg; phenylalanine 40 mg; Mn sulphate 0.40 gm; zinc sulphate 0.15 gm; iron sulphate 0.50 gm; copper sulphate 0.04 gm; cobalt chloride 0.01 gm.

### Estimation of blood parameters

At day 15<sup>th</sup>, and 30<sup>th</sup> of age, blood samples from five birds in each replicate randomly were collected from the bronchial vein in a test tube with EDTA anticoagulant. Hematological parameters such as RBCs, WBCs, PCV, Hb, together were determined by routine methods as previously described (Al-Daraji, 2008).

### Estimation of antibody titer by ELISA

On day 35<sup>th</sup> of age, blood samples were collected from five birds in each replicate from the bronchial vein in a test tube without anticoagulant. The blood allowed to clot and centrifuged for 10 minutes at 3000 rpm to obtain on serum which stored in deep freeze (-20) until analysis (Al-Daraji, 2008). Serum was performed according to the manufacturer's instructions listed in the Proflok ELISA Kit (Synbiotics–USA), which is a rapid serological test for the detection of antibody in chicken serum samples.

### Statistical analysis

Data generated from experiment was carried out in a complete randomized design (Steel and Torrie, 1980). These data were subjected to ANOVA according to general linear model procedure of SPSS software (SPSS, 2001). The significant differences among means were determined by Duncan's multiple range tests with  $p < 0.05$  level of significance.

## RESULTS & DISCUSSION

The effect of supplementation ration with thyme and garlic on antibody titers against ND virus at 15<sup>th</sup> and 30<sup>th</sup> day-old chicks was presented in (Table 2). Result revealed that antibody titers were increased significantly ( $p < 0.05$ ), in T4 (chicks fed basal diet supplemented with 1% thyme and 1% garlic) and T3 (chicks fed basal diet supplemented with 1% garlic) then T2 (chicks fed basal diet supplemented with 1% thyme)

respectively as compared with control group at 15<sup>th</sup> and 30<sup>th</sup> day-old chicks.

**TABLE 2:** Mean  $\pm$  standard errors for antibody titers against Newcastle disease of birds during the experimental period

Treatment \ Age	T1 (control)	T2 Thyme 1%)	T3 (Garlic 1%)	T4 (Garlic 1% and Thyme 1%)
15 days	2402 $\pm$ 7.10 c	2997.35 $\pm$ 10.04 b	3002.12 $\pm$ 22.2 b	3139.06 $\pm$ 42.00 a
30 days	1221.13 $\pm$ 30.01d	1930 $\pm$ 20.43c	1884.80 $\pm$ 43.14b	2140.53 $\pm$ 23.74a

Means with different letters in the same row differed significantly (P <0.05)

The effect of supplementation ration with thyme and garlic on some blood parameters were presented in (Table 3) and result revealed that RBCs, PCV, Hb, and WBCs were increased significantly (p 0.05), in T4 (chicks fed basal diet

supplemented with 1% thyme and 1% garlic) and T3 (chicks fed basal diet supplemented with 1% garlic) then T2 (chicks fed basal diet supplemented with 1% thyme) respectively as compared with control group.

**TABLE 2:** Effect of ration supplementation with different levels of fish oil on RBCs, WBCs, PCV and Hb

Parameter \ Treatment	T1 (control)	T2 Thyme 1%)	T3 (Garlic 1%)	T4 (Garlic 1% and Thyme 1%)
RBCs (x10 <sup>6</sup> /mm <sup>3</sup> )	3.90 $\pm$ 0.59b	4.61 $\pm$ 0.69ab	4.93 $\pm$ 0.74a	5.63 $\pm$ 0.74a
PCV (%)	29.08 $\pm$ 0.85b	33 $\pm$ 0.88ab	35.33 $\pm$ 0.88a	35.89 $\pm$ 0.88a
Hb (mg/dl)	9.03 $\pm$ 0.36c	11.59 $\pm$ 0.25b	11.92 $\pm$ 0.42b	13.18 $\pm$ 0.42a
WBCs (x10 <sup>3</sup> /mm <sup>3</sup> )	20 $\pm$ 1.03c	23.01 $\pm$ 0.49b	22.96 $\pm$ 0.96b	24 $\pm$ 0.96a

Means with different letters in the same row differed significantly (P <0.05)

Thymol and carvacrol from (thyme) and allicin from (garlic) are active material in these plants, which are considered as appetizer and stimulating of digestion, in addition to their antimicrobial activity against intestinal bacteria resulting of enhancing health status and growth. Improvement titer of antibody against ND viruses could be due to their relationship between ND vaccine and immunomodulator feed additive (medicinal plant) or present of active ingredients in thyme and garlic. Thymol, carvacrol, allicin could have positive effects on performance and growth of broilers also antibacterial, anticoccidial, antifungal, and antioxidant effects. These growth promoter feed additives relieve the host animal from immune defense during stress and critical situations also arise the intestinal availability for absorption of nutrients and assist the animal to grow better. So that the highest antibody titers were seen significantly (p 0.05) in T4 (chicks fed basal diet supplemented with 1% thyme and 1% garlic) as compared with other groups at 15<sup>th</sup> and 30<sup>th</sup> day-old chicks may be due to interaction between dietary inclusion and synergistic effect of two plants thyme and garlic. Josling, (2001) reported that garlic mobilized immune system and empowers the defense ability of the body against infectious organisms. Our results were in agreement with some studies (Jameel, 2008; Al-Kassie and Jameel, 2009; Windisch *et al.*, 2008; Najafi and Torki, 2010; Mansoub and Nezhady, 2011) who reported that these plants improving health status and performance of broiler, in addition to improve antibody titer against ND virus. Garlic has been used for about 50 years as antibiotic growth promoters and to enhance growth performance in poultry (Dibner and Richards, 2005; Demir *et al.*, 2008). Garlic is well known as a spice and herbal medicine for the prevention and treatment of a variety of diseases ranging from infections to heart diseases, antimicrobial activity, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and cardiovascular-protecting effects (Reuter *et al.*, 1996, Adibmoradi *et al.*, 2006, Javandel, 2008). Garlic

stimulates the NK cells (Burger *et al.*, 1993) and it increases the alkaline-phosphatase enzyme activity (Platel *et al.*, 2004).

The hematological values obtained in this study indicated that the means of total red blood cells, packed cells volume, total white blood cells and Hb were significantly higher (p 0.05) in T4 (chicks fed basal diet supplemented with 1% thyme and 1% garlic) then T3 and T2 as compared with control group. These differences could be attribute to interaction between dietary inclusion and synergistic effect of two plants thyme and garlic. Our results were consistent with several researches (Jameel, 2008; Al-Kassie and Jameel, 2009; Al-Kassie, 2009) which reported that using thyme and cinnamon in broilers diet, was significantly increased RBC, HCT, Hb and WBC values as compared with the control group. On the other hand, results of the present study was disagreement with Elagib *et al.*, (2013) who reported that no significant effect (P>0.05) was detected on total red blood cells, packed cells volume, total white blood cells and the differential count of white blood cells including neutrophile, eosinophile, monocytes and lymphocytes. In conclusion, the results suggest that supplementing broilers diet with mixture of 1% thyme plus 1% garlic could enhance the immune response and blood profile of broilers. These effects may be due to interaction between dietary inclusion and synergistic effect of two plants thyme and garlic.

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