

## Effect of Dietary Onion, Garlic, Red Pepper and Anise as Natural Feed Additives on Some Hematological Studies of Japanese Quail Chicks

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### Abstract

A total number of 300 quail chicks one week old were randomly divided into 5 treatments groups (60) chicks each group was subdivided into 3 replicates of 20 chicks each to study the effect of some natural feed additives such as onion powder, garlic powder, red pepper, and anise as natural feed additives on some productive responses of Japanese quail chicks. Birds fed 5 experimental dietary groups as follows:- the 1st group was fed on a basal diet without feed additives (control), the 2nd group was fed on a basal diet supplemented with 0.5% onion powder, the 3rd group was fed on a basal diet supplemented with 0.5% garlic powder, the 4th group was fed on a basal diet supplemented with 0.5% hot pepper, and the 5th group was fed on a basal diet supplemented with 0.5% anise. The experimental treatments started from 2 weeks old until 6 weeks of age. After 5 weeks from beginning of the experiment, 3 chicks from each treatment were injected intravenously in brachial vein with 0.2 ml of 10% suspension of sheep red blood cells to determine the activity of antibody. Three birds within each treatment were randomly selected and sacrificed, after six weeks from beginning of the treatments. Blood samples were collected in tubes contained heparin as an antic agglutination substance to study blood physical characteristics. The data revealed that, the greatest numerically improvement ( $P>0.05$ ) in packed cell volume and MCV was recorded for birds fed dietary onion. Birds dietary anise presented the best ( $P>0.05$ ) values of MCH compared with other dietary treatments. Birds fed dietary garlic and anise recorded the greatest ( $P<0.01$ ) values of total immunoglobulin compared with all dietary groups. The greatest ( $P<0.01$ ) values of total immunoglobulin were recorded for birds fed dietary garlic and anise compared with all dietary groups. No significant ( $P>0.05$ ) difference was detected in the values of Heterophil %, lymphocyte %, Heterophil : lymphocyte (H: L) ratio %, monocyte %, Eosinophils %, and basophil % of Japanese quail birds fed all dietary treatments.

**Keywords:** Blood Characteristics; Natural Feed Additives; Quail Chicks

### Introduction

In poultry nutrition, it is a solid fact that feeding cost is considered the most expensive item in the whole production process. Therefore, attempts are usually made to reduce feed cost without adversely affecting performance or product safety. Feed additive are classified into two categories: 1st those essential for the biological function of birds as in vitamins and trace elements, whereas, the 2nd category includes additives not essential for biological function, but have demonstrated a positive effect on bird's as in growth promoters, absorption enhancers, anti-microbial agents and metabolic modifiers [1-6]. As a result, supplementing broiler diets with some feed additives may be considered as an alternative to improve growth and feed conversion efficiency.

The use of antibiotic growth promotants (AGP) to improve animal performance has been practiced during last 50 years. However, when animals are exposed to low levels of antibiotics, resistant cells survive and grow producing antibiotic-resistant populations.

Consequently, the use of AGP in food animals has been banned in the European Union [7] and many other countries. In this context, alternatives to AGP are of importance. Most supplements which use as alternatives to AGP have effects on gut microflora, either directly or indirectly [8]. Using Herbs, spices, and various plant extracts have received increased attention as possible alternatives to AGP, since they are considered as natural products [9].

Medicinal and aromatic plants are cultivated in large areas in Egypt, about 48 thousands feddans were cultivated with medicinal and aromatic plants in Egypt [10]. Aromatic plants and essential oil extracted from them have been used as alternatives to antibiotics. For this reason, these plants are becoming more important due to their growth promoting and antimicrobial effect on animal health [11]. Medicinal plants has an effective compounds in their tissues including phenols and polyphenols, terpenoids and essential oils, alkaloids, lectins and polypeptides and other compounds applied antibacterial and immune stimulation effects [12] digestive pro-

cess stimulation [13], reducing levels of blood fat and cholesterol [14], antioxidant property [15] and finally the growth stimulator [16]. Feed additives such as onion, garlic, red pepper and anise. Onion has the properties of strengthening the immune system. Previous studies suggest that the biological properties of onion are widely related to sulfur-containing compounds [17]. Moreover, garlic has several beneficial effects on both humans and animal having antimicrobial, antioxidant properties [18]; antiviral [19] and antifungal [20].

Therefore, the present study conducted to speculate the effects of adding some natural feed additives such as, dried onion (*Allium cepa*), dried garlic (*Allium sativum*), red pepper (*Capsicum annum*. L), and anise (*Pimpinella anisum*) at the levels of 0.5% each to growing Japanese quail diet to enhance some productive responses.

## Materials and Methods

This study was carried out at the farm of Animal and Poultry Production, Faculty of Agriculture, Minia University, during the period from 28 May to 27 June 2016. It was designed to study the effect of some herbal feed additives such as, onion powder, garlic powder, red pepper and anise as natural feed additives on some productive and physiological response of Japanese quail chicks.

### Experimental birds

A total number of 300 quail chicks one week old were randomly divided into 5 treatments groups (60) chicks each. Each group was subdivided into 3 replicates of 20 chicks each. The birds were housed in an open house in cleaned and fumigated battery cages (100x70x20). Feed and were offered to the birds during the experimental periods (2-6 weeks of age) ad-libitum. All chicks were reared under similar hygienic and environmental condition.

### Experimental diets

Birds of the all experimental groups were fed on commercial basal diet (25% CP and 2945 Kcal ME/Kg diet) according to National Research Council, NRC, (1994) as follows:- The 1<sup>st</sup> group was fed on a basal diet without feed additives (control), the 2<sup>nd</sup> group was fed on a basal diet supplemented with 0.5% onion powder, the 3<sup>rd</sup> group was fed on a basal diet supplemented with 0.5% garlic powder, the 4<sup>th</sup> group was fed on a basal diet supplemented with 0.5% hot pepper and the 5<sup>th</sup> group was fed on a basal diet supplemented with 0.5% anise. The formulation and proximate analysis of the experimental basal diets (percent as fed) reported in table 1.

Premixes contributed the following nutrients per kilogram of complete feed: vitamin A, 2,300 IU; vitamin D3, 400 IU; vitamin E, 1.8 mg; vitamin B12, 3.5 mg; riboflavin, 1.4 mg; panthotenic acid, 2 mg; nicotinic acid, 7 mg; pyridoxine, 0.25 mg; folic acid, 0.15 mg; menadione, 0.3 mg; thiamin, 0.15 mg; manganese oxide, 35 mg; ferrous sulfate 35 mg; zinc oxide, 30 mg; copper sulfate, 60 mg; cobalt carbonate, 5 mg; potassium iodine, 0.6 mg; selenium vanadate, 0.09 mg. Based on NRC [21] feed composition table.

Ingredients	Basal diet %
Grain sorghum	55.05
Soybean meal	29.85
Gluten	10.00
Carbonate calcium	1.55
Lysine	0.19
Molasses	2.00
Vegetable oil (corn)	0.16
Common salt	0.25
Premix	0.30
Total	100.00
Calculated Chemical analysis	
Crude protein	25
Metabolizable energy (Kcal/kg)	2945 Kcal/Kg
Crude fiber	2.42
Ether extract	2.82

**Table 1:** Formulation and calculated proximate analysis of the experimental basal diets.

### Preparation of feed additives

Onion (*Allium cepa*) and garlic (*Allium sativum*) bulbs were peeled and carried out at laboratory of Animal Production, Faculty of Agriculture, Minia University, grated into smaller pieces and air-dried. The dried garlic and onion were separately pulverized and stored in cellophane bags until required for use. The Red pepper (*Capsicum annum*. L) and anise (*Pimpinella anisum*) were purchased from local market, ground separately to a fine powder and then added in different mixture levels to basal diets resulting in five experimental groups.

### Measurements

#### Collection of blood samples

After 5 weeks from beginning of the experiment, 3 chicks from each treatment were injected intravenously in brachial vein with 0.2 ml of 10% suspension of sheep red blood cells to determine the activity of antibody. Three birds within each treatment were randomly selected and sacrificed, after six weeks from beginning of the treatments. Blood samples were collected in tubes contained heparin as an antic agglutination substance to study blood physical characteristics.

### Hematological studies

#### Total erythrocytic count ( $N \times 10^6$ )

The total red blood corpuscles (RBC's) were counted using the double improved Neubauer chamber as described by (Daice and Lewis, 1991). The total number of cells per mm<sup>3</sup> of blood was calculated using the following equation:

Total number of RBCs/mm<sup>3</sup> blood =  $N \times 4000 \times 200 / 5 \times 16 = N \times 10^6$   
Where "N" is the mean erythrocyte count in five large squares.

### Hemoglobin percentage (Hb. %)

Hemoglobin concentration (gm/dl) was determined using cyanomethemoglobin method. In this method, all haemoglobin derivatives except sulphaemoglobin are converted to methemoglobin using ferricyanide and cyanide ions. Methemoglobin is a stable red compound that can be measured at 540 nm (Campbell., 1995).

### Microhaematocrit, packed cell volume, (PCV %)

The well mixed blood was drawn into a Microhaematocrit tube 7.5 cm long, 1mm internal diameter and one end was sealed with clay. The tube was then centrifuged in a Microhaematocrit centrifuge for 5 minutes at 15,000 rpm. The readings were made with the aid of a Microhaematocrit reader (Dacie and Lewis, 1991).

### Wintrobe erythrocyte indices

Calculation of the absolute values or the erythrocyte indices, namely mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the following equations, according to [22].

$$MCV (f1.) = \frac{Hct. (ml/dl)}{RBC's (million/ml)} \times 10$$

$$MCH (Pg.) = \frac{Hb. (g/dl)}{RBC's (million/ml)}$$

$$MCHC (g/ dl) = \frac{Hb. (g/dl)}{Hct. (ml/dl)}$$

Where F1 = (femtoliter) = 10<sup>-15</sup> Liter, Hb = Hemoglobin %, Hct = Hematocrit %, Pg = (pictogram) = 10<sup>-12</sup> gram and RBC's = Number of Red Blood Cells eighed and expressed as a percentage of live body weight.

## Result and Discussion

### Effect of dietary feed additives on hematological studies

The effects of dietary feed additives supplementation (onion, garlic, red pepper and anise) at the level of 0.5% for each addi-

tion on (RBC's Nx10<sup>6</sup>/micro liter, PCV%, Hb mg/dl, MCV, MCH and MCHC) of Japanese quail at the end of the experiment (6 weeks of age) are presented in Table 2 and Figures 1 and 2. The data performed that, no significant (P>0.05) difference was detected in all hematological parameters studied (RBC's Nx10<sup>6</sup>/micro liter, PCV%, Hb mg/dl, MCV, MCH and MCHC) among dietary treatments. Whereas, there was a slightly numerically improvement (P>0.05) in red blood cells count, hemoglobin and MCHC for birds fed dietary garlic. Hence, the greatest numerically improvement (P>0.05) in packed cell volume and MCV was recorded for birds fed dietary onion. Birds dietary anise presented the best (P>0.05) values of MCH compared with other dietary treatments.



**Figure 1 and 2:** Effect of dietary feed additives supplementation on some hematological parameters of growing Japanese quail.

The slightly improvement in RBC's as effect of adding garlic and anise may be due to it has bioactive components like sulfur containing compounds (Alliin, Diallylsulfides and Al-licin) that act as antibacterial, antifungal, anti-parasite, antiviral, antioxidant, anti-thrombotic, ant cancerous and vasodilator characteristics. These results are going well with those observed by Ademola., *et al.* [23]

Items	Treatments (0.5% of each additive)					SE	Significance
	Control	Onion	Garlic	Red pepper	Anise		
RBC`s n x 10 <sup>6</sup> /micro liter	5.54	5.12	6.33	5.00	5.28	0.31	NS
Hemoglobin %	10.20	9.60	11.13	8.61	10.54	0.74	NS
PCV%	42.00	42.66	42.50	34.66	35.33	3.41	NS
MCV*10 <sup>-5</sup> (fl)	75.67	85.22	50.69	68.89	66.88	9.16	NS
MCH*10 <sup>-5</sup> (pg)	1.84	1.90	1.76	1.71	2.00	0.12	NS
MCHC (g/dl)	2.44	2.25	4.65	2.50	3.02	0.93	NS

**Table 2:** Effect of dietary feed additives supplementation on some hematological parameters of growing Japanese quail.

NS= Not significant; ±SE= standard error.

reported that, dietary garlic (1, 1.5 and 2%) did not effect on the PCV, Hb and RBC concentration of the broiler chickens. Moreover, Elagib, *et al.* [24] found that, broiler chicks fed different levels of garlic (0.3 and 5%) had no significant effect ( $P>0.05$ ) on packed cells volume and total red blood cells of birds.

**Effect of dietary feed additives on some immunological studies**

The effects of dietary feed additives supplementation (onion, garlic, red pepper and anise) at the level of 0.5% for each addition on Heterophil %, lymphocyte %, Heterophil: lymphocyte (H:L) ratio %, immunoglobulin (IG ) monocyte %, Eosinophils %, and basophil % of Japanese quail at the end of the experiment (6 weeks of age) are presented in Table 3 and Figures 3 and 4. The data re-

vealed that, there was a highly significant difference ( $P<0.01$ ) in values of total immunoglobulin between all dietary treatments. The greatest ( $P<0.01$ ) values of total immunoglobulin were recorded for birds fed dietary garlic and anise compared with all dietary groups. No significant ( $P>0.05$ ) difference was detected in the values of Heterophil %, lymphocyte %, Heterophil : lymphocyte (H:L) ratio %, monocyte %, Eosinophils %, and basophil % of Japanese quail birds fed all dietary treatments. Birds fed dietary garlic recorded the best non-significant ( $P>0.05$ ) values of Heterophil and H:L ratio compared with all dietary groups. While, birds fed dietary anise, control and onion recorded the greatest numerically ( $P>0.05$ ) enhancement in the values monocyte %, Eosinophils %, and basophil % respectively compared with other dietary groups.

Items	Treatments (0.5% of each additive)					SE	Sig.
	Control	Onion	Garlic	Red pepper	Anise		
Heterophils %	16.55	18.39	21.06	18.56	17.02	3.24	NS
Lymphocytes %	53.06	52.94	43.99	56.30	48.49	6.53	NS
H/L Ratio	31.03	34.56	48.64	33.81	38.56	9.26	NS
Total immunoglobulin	868.79 <sup>b</sup>	931.39 <sup>b</sup>	1152.89 <sup>a</sup>	978.16 <sup>b</sup>	1120.85 <sup>a</sup>	25.55	**
Monocytes %	16.00	14.21	15.80	11.22	16.84	4.54	NS
Eosinophils %	8.50	5.88	8.14	7.14	8.47	1.82	NS
Basophiles %	5.87	11.76	11.00	6.76	9.16	3.12	NS

**Table 3:** Effect of dietary feed additives supplementation on some immunological studies of growing Japanese quail.

a-b Values within columns with no common superscripts are significantly different ( $p\leq 0.05$ ).

NS= Not significant;  $\pm$ SE= standard error; \*\* =  $P \leq 0.01$

**Figure 3:** Effect of dietary feed additives supplementation on Heterophil s% Lymphocytes % and H/L Ratio of growing Japanese quail.

**Figure 4:** Effect of dietary feed additives supplementation on Monocytes % Eosinophils %and Basophiles % of growing Japanese quail.

The enhancement on immunoglobulin parameters as a result of feeding Japanese quail birds on some medical herbs may be due to that the aromatic plants is one of the ban herbal plants played an important role in maintaining human health and becoming more important due to their antimicrobial, stimulating effects on birds digestive system through increasing their digestive enzymes and improve utilization of digestive products through enhancing liver function [9]. Aromatic plants have been used traditionally in the therapy of some diseases for a long time in the world [25]. 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 [26], antibacterial [27], antifungal [28] and antipyretic [29].

The present results are going well with the finding of, Yang, *et al.* [30] observed that, there were no significant difference ( $P \geq 0.05$ ) on broiler chicks white blood cells including neutrophile, Eosinophils, Monocytes and lymphocytes when birds fed different levels (0%, 0, 3 and 5%) of garlic supplementation. Also, Mohebbifar and Torki [31] found that, no affected of garlic supplementation (200mg/kg diet) on heterophils in of Ross broilers chicken. Furthermore, Seyed, *et al.* [32] found that there was no significant difference in heterophyles ratio to lymphocytes H/L and white blood cells concentration due to supplemented broiler chicks with garlic supplementation (1%). Kolawole, *et al.* [33] found that, The leukocyte or white blood cells (WBC) of broilers fed control diet and diet with 0.1% dried hot red pepper (DHRP) were similar and significantly lower than those fed diets with 0.2 and 0.3% DHRP meal.

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