Afro-Asian Journal of Scientific Research (AAJSR)
المجلة الأفروآسيوية للبحث العلمي (AAJSR)
E-ISSN: 2959-6505
Volume 1, Issue 4, October-December 2023, Page No: 241-253
Website: https://www.aajsr.com
SJIFactor 2023: 3.908 معامل التأثير العربي (AIF) 2023: 0.51 ISI 2023: 0.337

Impact of Ginger and Propolis-Enriched Diets on the Immune System of Japanese Quail: An In-depth Analysis

Alallam Belqasem Alallam Abdulhamid[1*], Buthuynah Massoud Alraeboub[2], Ramadan Daw Mohamed[3], Ammnah Salim Qareerah[4]
[1,2,3] Department of Animal Production, Faculty of Agriculture, Bani Waleed University, Bani Walid, Libya
[4] Department of Biology, Faculty of Education, Bani Waleed University, Bani Walid, Libya

*Corresponding author: alam.algmle@gmail.com

Received: September 30, 2023	Accepted: November 23, 2023	Published: November 30, 2023

Abstract:
The immune system plays a vital role in protecting organisms from various infectious agents. In recent years, there has been growing interest in the potential of natural products to enhance immune function. This study aimed to investigate the impact of ginger and propolis-enriched diets on the immune system of Japanese quail. A total of 100 quails were divided into four groups and fed with different diets: control diet, ginger-enriched diet, propolis-enriched diet, and a combination of ginger and propolis-enriched diet. The immune parameters were assessed, including antibody production, phagocytic activity, and cytokine levels.
This study provides compelling evidence that the inclusion of ginger and propolis in the diets of Japanese quail has a positive impact on their immune system. The enhanced antibody production, phagocytic activity, and increased levels of IL-2 and IFN-γ suggest improved humoral and cellular immune responses.
Results indicated that quails fed with ginger and propolis-enriched diets exhibited enhanced immune responses compared to those on the control diet. The antibody production, measured through enzyme-linked immunosorbent assay (ELISA), showed a significant increase in the ginger and propolis-enriched diet groups. Additionally, the phagocytic activity of immune cells was significantly higher in the treatment groups. Cytokine analysis revealed elevated levels of interleukin-2 (IL-2) and interferon-gamma (IFN-γ) in the ginger and propolis-enriched diet groups, indicating enhanced cellular immune responses. These findings highlight the potential of ginger and propolis as natural immunomodulators for poultry.

Keywords: Immune System, Ginger, Propolis, Enriched Diets, Japanese Quail.

Cite this article as: A. B. A. Abdulhamid, B. M. Alraeboub, R. D. Mohamed, A. S. Qareerah, "Impact of Ginger and Propolis-Enriched Diets on the Immune System of Japanese Quail: An in-depth Analysis," *Afro-Asian Journal of Scientific Research (AAJSR),* vol. 1, no. 4, pp. 241–253, October-December, 2023.

تأثير الأنظمة الغنية بالزنجبيل وصمغ النحل على الجهاز المناعي لطائر السمان الياباني: تحليل في العمق

العلام بالقاسم العلام عبد الحميد[1*]، بثينة مسعود الرعبوب[2]، رمضان ضوء محمد[3]، آمنة سليم قريرة[4]
[1،2،3] قسم الإنتاج الحيواني، كلية الزراعة، جامعة بني وليد، بني وليد، ليبيا
[4] قسم الأحياء، كلية التربية، جامعة بني وليد، بني وليد، ليبيا

الملخص
يلعب الجهاز المناعي دوراً حيوياً في حماية الكائنات الحية من العوامل المعدية المختلفة. في السنوات الأخيرة، كان هناك اهتمام متزايد بإمكانيات المنتجات الطبيعية لتعزيز وظيفة المناعة. هدفت هذه الدراسة إلى دراسة تأثير النظام الغذائي الغني بالزنجبيل والبروبوليس على الجهاز المناعي لطائر السمان الياباني. تم تقسيم ما مجموعه 270 طائر السمان إلى
أربع مجموعات وتم تغذيتها بأنظمة غذائية مختلفة: النظام الغذائي المسيطر، والنظام الغذائي الغني بالزنجبيل، والنظام الغذائي الغني بالبروبوليس، ومزيج من النظام الغذائي الغني بالزنجبيل والبروبوليس. تم تقييم المعلمات المناعية، بما في ذلك إنتاج الأجسام المضادة، والنشاط البلعمي، ومستويات السيتوكينات.
تقدم هذه الدراسة أدلة دامغة على أن إدراج الزنجبيل وصمغ العسل في النظام الغذائي لطائر السمان الياباني له تأثير إيجابي على جهاز المناعة لديهم. يشير الإنتاج المعزز للأجسام المضادة، ونشاط البلعمة، وزيادة مستويات *IL-2* و*IFN-γ* إلى تحسين الاستجابات المناعية الخلطية والخلوية.
أشارت النتائج إلى أن طيور السمان التي تم تغذيتها بالزنجبيل والوجبات الغذائية الغنية بالدنج أظهرت استجابات مناعية معززة مقارنة بتلك الموجودة في النظام الغذائي المتحكم. أظهر إنتاج الأجسام المضادة، الذي تم قياسها من خلال مقايسة الامتصاص المناعي المرتبط بالإنزيم (*ELISA*)، زيادة كبيرة في مجموعات النظام الغذائي الغنية بالزنجبيل والبروبوليس. بالإضافة إلى ذلك، كان نشاط البلعمة للخلايا المناعية أعلى بكثير في مجموعات العلاج، كما كشف تحليل السيتوكين عن مستويات مرتفعة من الإنترلوكين 2 (*IL-2*) والإنترفيرون جاما (*IFN-γ*) في مجموعات النظام الغذائي الغنية بالزنجبيل وصمغ النحل، مما يشير إلى الاستجابات المناعية الخلوية المحسنة، وبالتالي تسلط هذه النتائج الضوء على إمكانات الزنجبيل وصمغ النحل كمعدلات مناعية طبيعية للدواجن.

الكلمات المفتاحية: نظام المناعة، الزنجبيل، صمغ النحل، طائر السمان الياباني.

Introduction

The immune system plays a vital role in maintaining the health and well-being of animals, including poultry. In recent years, there has been growing interest in using natural feed additives to enhance immune function and overall performance in poultry [1]. Antibiotics have revolutionized the field of medicine by providing effective treatments against various bacterial infections. These remarkable compounds are microbial metabolites produced by fungi and algae. Antibiotics possess low molecular weight and exhibit the ability to inhibit the growth and reproduction of other microorganisms, even at low concentrations. In this article, we will explore the fascinating world of antibiotics, their sources, and their essential role in combating infectious diseases [2].

Zingiber officinale as an Immunomodulatory Agent is a well-known medicinal plant with various bioactive compounds that possess immunomodulatory properties. It contains gingerol, shogaol, and other phenolic compounds that have been reported to exhibit antioxidant and anti-inflammatory effects. These properties make ginger a promising candidate for improving immune function in Japanese quail [3]. Propolis is a resinous substance collected by bees from plant sources. It has been used in traditional medicine for its antimicrobial and immunomodulatory properties. Propolis contains a wide range of bioactive compounds, including flavonoids, phenolic acids, and terpenoids, which contribute to its immunomodulatory effects. A study by Bankova et al. (2014) highlighted the immunomodulatory potential of propolis, showing its ability to enhance immune parameters in animals and humans [4]. Japanese quail (Coturnix japonica) is a commonly used avian model for studying various aspects of physiology and immunology. Several studies have explored the effects of ginger and propolis on the immune system of Japanese quail, providing valuable insights into their immunomodulatory properties. A study by Samadi et al. (2017) investigated the effects of ginger extract on immune parameters in Japanese quail. The results indicated that ginger supplementation enhanced humoral and cellular immune responses, including increased antibody titers and improved lymphocyte proliferation [5].

b. In another study by Abdel-Moneim et al. (2018), the researchers evaluated the immunomodulatory effects of propolis in Japanese quail. The findings demonstrated that propolis supplementation improved immune parameters, including increased phagocytic activity and enhanced lymphocyte proliferation [6].

The immunomodulatory effects of ginger and propolis can be attributed to their bioactive components. Ginger compounds, such as gingerol and zingerone, exhibit antioxidant and anti-inflammatory properties, which may contribute to immune system modulation. Similarly, propolis bioactive compounds, especially flavonoids, have been shown to stimulate immune cells, modulate cytokine production, and enhance phagocytic activity.

Animals, poultry in particular, are very sensitive to pathogenic bacteria such as Escherichia coli, and Salmonella sp. Clostridium perfringens and Campylobacter sputorum. The pathogenic microbial flora in the small intestine competes with the host for nutrients while at the same time inhibiting the binding of the bile acids to the pertinent substances, they decrease the digestion of fats and fat-soluble vitamins.

This leads to a decrease in performance and an increase in disease rate. Antibiotics, which have been used as growth promoters in poultry feed for a long time, improve growth performance by stabilizing the microbial flora in the intestine and preventing some specific intestine pathogens [7]. Antibiotics are low-molecule-weight microbial metabolites that are produced by fungi and algae that can, even at low concentrations, prevent the growth of other bacteria [8]. Antibiotics are microbial metabolites produced by fungi and algae which have low molecule weight and can inhibit the growth of other microorganisms even in low concentrations [9]. While antibiotics have prevalently been used as growth promoters in animal nutrition, the European Community has prohibited the use of antibiotics in animal nutrition as growth promoters from January 1, 2006 [10]**.** As a result of the ban on antibiotic growth promoters due to the demands from medicine and consumers; explorations on alternative products have started. Consequently, studies on natural products such as plant extracts have recently gained great attention [10].

Material and methods

a. Sample collection and histological evaluation:

After the sacrifice, the livers, testes, spleen and bursa of Fabricius were dissected out and weighed using Metler's Analytical Balance. Specimens from these organs were collected and then fixed in 10% buffered formalin solution for 24 hrs., followed by washing in water. Later on, specimens were dehydrated through a graded series of ethanol (50%, 70%, 90%, and 100%), clearing with xylene, embedding and blocking in paraffin. Sections (5-6 µm in thickness) were cut and stained with hematoxylin and eosin (H&E) according to Junqueira and Carneiro, (2003). The tissue sections were then examined under the light microscope and some photomicrographs were captured from the present microscopic changes in the examined organs (livers, testes, spleen and bursa of Fabricius) of various groups. Thereafter, the following factors were measured and evaluated histomorphometrically in the testes of both, control and treated groups using a calibrated eyepiece micrometer:

1. The total number of seminiferous tubules/mm^2.
2. The diameter of each of 10 randomly selected circular or nearly circular profiles of seminiferous tubules in various groups [µm].
3. The epithelial height in each of also 10 randomly selected circular or nearly circular profiles of seminiferous tubules [µm].

Table (1) shows the Total number of seminiferous tubules/mm^2.

Table1. Total number of seminiferous tubules/mm^2

G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
09	12	14	10	21	13	14	12	10	12
12	11	11	14	11	15	15	13	11	12
13	15	10	15	10	11	11	15	11	14
20	15	09	11	09	14	09	11	15	13
11	16	10	09	15	16	12	12	09	12
15	19	12	16	14	10	16	09	12	12
12	14	12	15	16	14	14	11	11	13
14	19	10	15	17	16	15	15	13	14
16	11	13	11	16	10	12	14	14	15
15	20	12	10	14	12	12	10	10	11

Table2. Diameter of circular or nearly circular seminiferous tubules [µm]

G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
230	190	200	180	150	250	265	195	195	245
210	200	220	150	215	260	200	200	225	270
280	160	150	180	200	215	235	180	230	210
240	205	140	175	190	220	230	195	195	200
290	150	175	155	120	220	240	200	210	205
220	230	225	180	180	210	215	225	200	200
215	225	195	185	175	195	220	230	205	195
200	200	215	225	200	200	240	200	200	220
210	175	190	200	190	225	250	180	220	215
220	190	230	180	180	265	210	205	215	245

Table3. Height of seminiferous tubular epithelium [µm]

G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
65	85	90	70	50	70	45	90	90	65
75	55	85	95	55	75	65	85	80	50
55	90	80	70	50	80	60	105	105	75
70	75	115	65	75	55	40	80	55	45
70	105	85	70	65	95	45	75	85	60
55	100	75	90	70	105	60	65	70	80
45	75	65	80	80	50	55	55	50	65
80	60	90	65	95	65	65	60	70	75
105	65	65	60	60	75	85	90	65	50
85	55	85	55	70	55	50	85	75	55

b. Experimental diets:

Each experimental group received one of the following dietary treatments through the growing period (1 to 5 weeks of age). In this study antibiotic (Tyrosin) and two natural feed additives, being ginger and propolis and their blend were used. The order of dietary treatments was as follows:

1. Basal diet only without supplementation (served as control).
2. Basal diet+ 100 mg antibiotic Tyrosin / kg diet.
3. Basal diet + 125 mg ginger /kg diet. (Ginger was obtained from Imtenan Health Shop, 20 Mohamed Bahaa ElDin ElGhatory, Semouha, Alexandria, Egypt).
4. Basal diet + 250 mg ginger /kg diet.
5. Basal diet + 500 mg propolis /kg diet. (Propolis obtained from A Chinese commercial propolis as powder from Egyptian market (NO. HNBOOMBP-01, ISO9001 by Sea, Code: 410004900, Henan Boom, China).
6. Basal diet + 1000 mg propolis /kg diet.
7. Basal diet + 125 mg ginger +500 mg Propolis /kg diet.
8. Basal diet + 125 mg ginger +1000 mg Propolis /kg diet.
9. Basal diet + 250 mg ginger +500 mg Propolis /kg diet.
10. Basal diet + 250 mg ginger +1000 mg Propolis /kg diet.

Table 4. Composition and calculated analysis of the basal experimental diets

Ingredients	%
Yellow corn	53.30
Soybean meal (44 %)	33.00
Concentrate (50 %) *	10.00
Di-calcium phosphate	0.20
Limestone	1.70
Sunflower oil	0.80
Vit. and min. mix.**	0.50
Salt (NaCl)	0.50
Total	100
Calculated analyses[1]:	
Crude protein, %	24.05
ME (Kcal/ Kg diet)	2907.10
Ether extract, %	2.44
Crude fiber, %	3.63
Methionine, %	0.76
Methionine + cysteine, %	0.88
Lysine, %	1.42
Calcium, %	1.11
Av. Phosphorus	0.39

*** Concentrate**: ME (K cal/kg) 2870, Crude protein 50%, Crude fiber 1.51%, Crude fat 1.54%, Calcium 4.29%, Phosphorus 2.39%, NaCl 0.8%, Methionine 4.6%, Methionine &Cysteine5.38%, Lysine 3.90%.
** Each kg of vitamin and minerals mixture contained: Vit. A, 4,000,000 IU ; Vit. D_3, 500,000 IU; Vit, E, 16.7 g., Vit. K, 0.67 g., Vit. B1, 0.67 g., Vit. B2, 2 g., Vit. B 6, .67 g., Vit. B12, 0.004 g., Nicotinic acid, 16.7 g., Pantothenic Acid, 6.67 g., Biotin, 0.07 g., Folic acid, 1.67 g., Choline chloride, 400 g., Zn, 23.3 g., Mn, 10 g., Fe, 25 g., Cu,1.67 g., I, 0.25 g.,Se, 0.033 g. and,Mg, 133.4 g.
[1] According to NRC (1994) [11].

c. Measurements of growing Japanese quail chicks:

1. Body weight and weight gain

Body weight was measured for all birds at the beginning of the experiment, and it was repeated weekly at the beginning of the week at the same time. Live weight gain was calculated by subtraction the live weight at the beginning of the week from the live body weight of the next week.

2. Relative growth rate:

Relative growth rate was estimated according to the equation of Broody [12].
Growth rate % = $(W_2 – W_1)$ X 100 / 0.5 $(W_1 + W_2)$
Where,
W_l = Body weight at the beginning.
W_2 = Body weight at the end of the period for which rate was calculated.

3. Feed consumption:

Weight of feed consumed (grams) per each replicate per period was calculated by subtracting the amount of feed left from that supplied.

4. Feed conversion ratio:

Feed conversion ratio was calculated as units of grams of feed consumed to produce one-unit body weight during the period according to this equation [11]:

$$\text{Feed conversion ratio} = \frac{\text{F. C.}}{\Delta \text{Wt}}$$

Where,

F. C. = Feed consumed during a period (g)
ΔWt = Weight gain within that period (g)

d. Carcass characteristics:

At the end of the experiment (5 weeks of age), four birds (2 males + 2 femaels) were randomly selected from each treatment, fasted for 12 hours, weighed, slaughtered by slitting the jugular vein of the birds, then scalded and defeathered. Carcasses were eviscerated manually and weighed. Liver, gizzard, spleen, heart, testes, bursa, and thymus were removed and separately weighed and the weights of these organs were expressed as a percentage of live body weight.

1. Physical characteristics of meat:

- **pH:** The pH value of meat was determined using pH meter according to the method of (Evans and Niven, 1960) [13].
- **Tenderness and water-holding capacity**: Tenderness and water-holding capacity were measured by Gray and Hamm method (1957) [14] as modified by Volovinskaia and Merkolova (1958) [15]. According to this method, 0.3 g of minced meat was put under an ashless filter paper (Whatman No.41) and pressed for 10 minutes using 1 Kg weight. Two-zones were formed on the filter paper and the surface areas were measured by planimeter. The internal zone due to the meat pressing indicated the tenderness in cm^2. The water-holding capacity was calculated by subtracting the area of the internal zone from that of the outer zone.
- **Color**

The color intensity of meat extract was determined according to the method mentioned by Yamazake (1981) [16]. Ten grams of meat sample were shaken with 22.5 ml distilled water in dark room for 10 minutes; the color intensity (absorbance) of filtrate was measured at 542 nm by spectrophotometer.

2. Digestibility trial

At 5 weeks of age, a digestibility trial was done using 30 males (three cocks per treatment) and each housed in individual metabolism cages which allowed a complete separation and collection of excreta and were assigned to each of the dietary treatment, for 5 consecutive days, to allow them become adjusted to the cage. Then excreta were quantitatively collected for a 4-d period during which feed consumption data were also recorded. The excreta were then dried in a forced air oven at 65º C for 24 hours. Following this, excreta were allowed to equilibrate in moisture with the air before being weighed, finely ground, and stored in glass bottles for analysis. Chemical analyses for nutrient were performed according to A. O. A. C. (1995) [17]. The faecal nitrogen was determined following the procedure outlined by Jakobsen *et al.* (1960) [18]; which enables the separation of urinary nitrogen from that of faecal nitrogen as following: A 70 ml of distilled water was added to 2 g of the dried excreta in 300 ml Beaker. A 20 ml of Sodium borate (dissolved 50 g boric acid + 100 g sodium hydroxide in 385 ml distilled water) and 6 ml. of potassium permanganate (dissolved 3.16 g potassium permanganate in 97 ml. of distilled water was added). The beaker was placed in water bath at a temperature of 50º C and stirred for an hour. It was left to settle for at least another one hour at room temperature. A 30 ml of 10% trichloro-acetic acid solution were added and stir with a glass rod. The beaker was left again for one hour at room temperature. Filter through 15-cm. ashless filter paper; wash 4 times with 25-30 ml of 2% tricholoro acetic acid for each time in order that the precipitate was free from solution by pressing it with plastic covered glass rod. The filter paper containing the sample (on the glass funnel) was dried in an oven at 90º C. The sample along with the filter paper was digested following the Kjeldahl method for determining the nitrogen. Digestibility was determined by accurately measuring feed intake and faecal output. Digestibility coefficient of crude protein, ether extract, crude fiber and nitrogen free extract was determined.

3. Blood hematological and biochemical characteristics:

Individual blood samples were taken from 2 birds from each sex within each treatment (on individual basis) at 5 weeks of age to determine the different hematological and biochemical characteristics. Blood samples were collected and divided into two equal parts. The first part of blood was collected on heparin as anticoagulant (0.1 ml of heparin to 1 ml of blood) according to Hawk *et al.* (1965) [19] to determine the blood hematology (white blood cells counts (WBCs), the differential of white blood cells, red blood cells (RBCs), hemoglobin concentration (Hb) and packed cell volume (PCV). The second part of each blood sample was centrifuged at 4000 rpm for 15 minutes to separate blood serum. The obtained serum was kept frozen at -18 °C until being analyzed. Serum samples were used for biochemical analysis using the specific kits.

Statistical analysis:

The differences among treatments were statistically analyzed by one-way ANOVA using SPSS® statistical software package for windows version 16.0. The significant differences between treatment means were separated by Duncan's Multiple Range-test.

Results and discussion

Live body weight and weight gain:

Results concerning the effect of dietary supplementation of antibiotic, ginger, propolis and the mixture of ginger and propolis on live body weight of growing Japanese quail are illustrated in Table 2.

Table 5. Effect of dietary Antibiotic, Ginger, Propolis and blend of antioxidant on live body weight (g) of Japanese quail during the period from 1 to 5 weeks of age.

Treatment mg/kg diet	Live body weight (g)				
Control	**W1**	**W2**	**W3**	**W4**	**W5**
Antibiotic 100 mg/kg	30.02±0.46	81.1±1.6	130.6±2.1	186.1±2.7	225.43±3.08
Ginger 125 mg/kg	30.0±0.46	84.2±1.4	133.6±2.1	189.8±2.7	227.64±3.81
Ginger 250 mg/kg	30.03±0.47	80.8±1.5	132.2±2.2	182.7±2.8	221.58±2.59
Propolis 500 mg/kg	30.04±0.47	81.7±1.5	131.7±2.4	183.3±2.7	222.80±3.26
Propolis 1000 mg/kg	30.01±0.44	80.0±1.3	134.1±2.0	184.9±2.7	227.44±3.63

Ginger 125 + Propolis 500 mg/kg	30.01±0.45	80.9±1.3	132.6±2.1	182.2±3.0	227.14±3.40
Ginger 125 + Propolis 1000 mg/kg	29.99±0.45	82.5±1.3	135.2±2.2	184.4±2.2	227.41±3.18
Ginger 250 + Propolis 500 mg/kg	29.99±0.44	81.8±1.3	133.4±2.0	181.9±2.0	222.03±3.24
Ginger 250 + Propolis1000 mg/kg	29.99±0.43	80.6±1.5	131.4±2.3	181.9±2.5	223.14±3.45
P value	30.04±0.49	80.0±1.4	131.8±2.1	181.8±2.6	224.25±3.42
	1.00	**0.592**	**0.924**	**0.482**	**0.850**

Results presented in Table (5) indicated that there were no significant differences in initial body weight among different treatments showing the random distribution of the experimental birds among treatments. Dietary supplementations did not significantly (P ≤ 0.05) influence the live weight of Japanese quail at 2, 3, 4 and 5 weeks of age.
Table (6) shows the effect of the dietary antibiotics, Ginger, propolis and a blend of antioxidants on body weight gain (g / bird /period) of Japanese quail during the period from 1 to 5 weeks of age.

Table 6. Effect of dietary antibiotic, Ginger, propolis and blend of antioxidants on body weight gain (g / bird /period) of Japanese quail during the period from 1 to 5 weeks of age.

Treatment mg/kg diet	**Bodyweight gain (g / bird)**			
	1-2w	**2-3w**	**3-4w**	**4-5w**
Control	51.09±1.38	49.52±1.32	55.51±1.52 ^{ab}	39.29±2.47
Antibiotic 100 mg/kg	54.20±1.13	49.40±1.10	56.17±1.34 ^{a}	37.56±2.87
Ginger 125 mg/kg	50.77±1.17	51.42±1.09	50.44±2.08 ^{c}	38.92±2.84
Ginger 250 mg/kg	51.62±1.26	50.07±1.19	51.55±1.17 ^{bc}	39.52±2.33
Propolis 500 mg/kg	49.96±1.06	54.10±1.06	50.83±1.29 ^{c}	42.54±2.23
Propolis 1000 mg/kg	50.91±1.04	51.72±1.10	49.60±1.61 ^{c}	44.90±2.17
Ginger 125 + Propolis 500 mg/kg	52.52±1.00	52.67±1.46	49.22±1.57 ^{c}	43.01±2.24
Ginger 125 + Propolis 1000 mg/kg	51.82±1.04	51.64±0.96	48.45±1.80 ^{c}	40.13±2.35
Ginger 250 + Propolis 500 mg/kg	50.57±1.23	50.81±1.25	51.20±1.37 ^{bc}	41.21±2.47
Ginger 250 + Propolis1000 mg/kg	49.96±1.17	51.76±1.06	50.02±1.39 ^{c}	42.47±2.40
P value	**0.268**	**0.137**	**0.004**	**0.569**

.
The results in Table (6) showed insignificant differences in body weight gain among different treatments through the periods 1-2, 2-3 and 4-5 weeks of age, however, significant (P ≤ 0.05) differences were recorded among different treatments through the period from 3-4 weeks of age. The best (P ≤ 0.05) value of weight gain was recorded in the group fed antibiotic containing diet as compared to the control group or the other experimental groups. On the other hand, the lowest values were recorded in the

group given ginger or propolis containing diets in comparison with the control group. When the data are pooled, the results showed also insignificant differences among different experimental groups. Numerical increase in body weight gain was recorded in the groups given 500 and 1000 mg propolis /kg diet, combination of 500 mg propolis plus 125 mg ginger in their diets and also antibiotic fed group. These groups surpassed the control one by 1.2, 0.9, 1.0 and 1.1 %, respectively. Table (6) shows the Effect of dietary antibiotic, Ginger, propolis and blend of antioxidant on body weight gain (g / bird /period) of Japanese quail during the period from 1 to 5 weeks of age

Figure (1) shows G1: Congested blood spaces (asterisk) with variable sized hepatocytic fatty vacuolations (arrows). H. and E. X= 400.

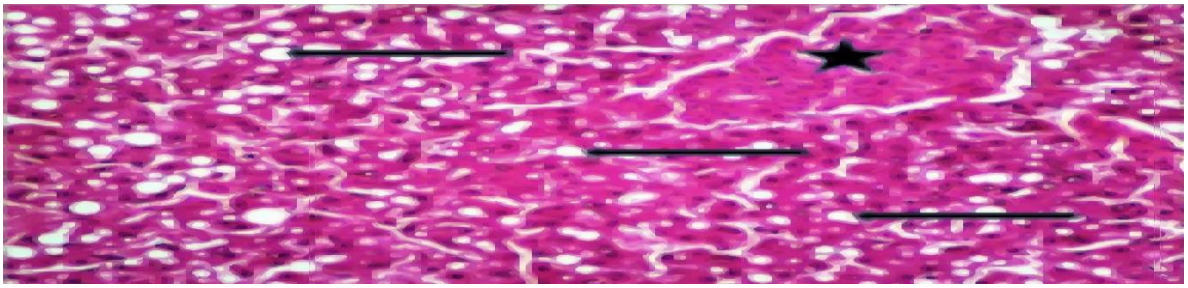

Figure 1. Liver, G1: Congested blood spaces (asterisk) with variable sized hepatocytic fatty vacuolations (arrows). H. and E. X= 400.

Figure (2) shows Liver, G2: Diffuse small sized hepatocytic fatty vacuolations (arrows).

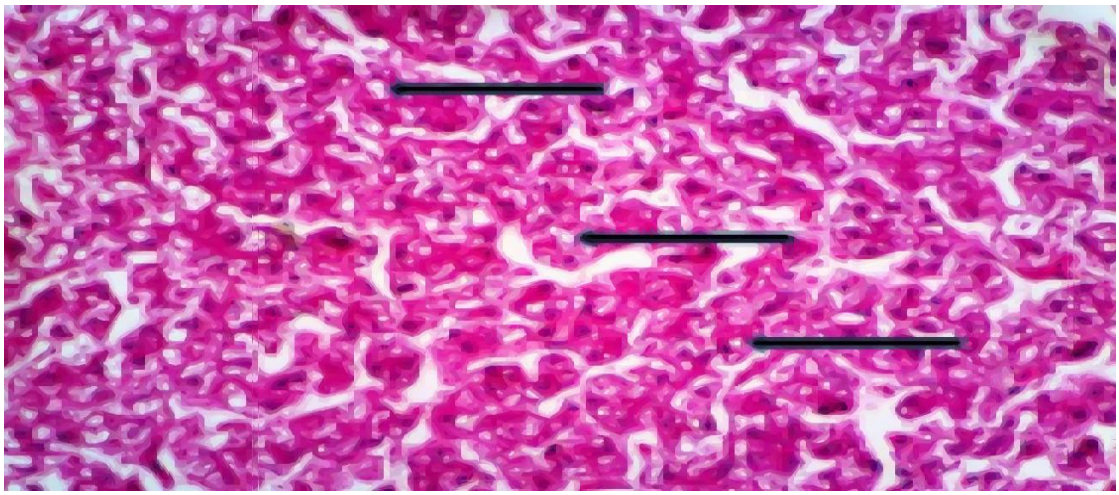

Figure 2. Liver, G2: Diffuse small sized hepatocytic fatty vacuolations (arrows). H. and E. X= 160

Figure 2. shows Liver, G3: Congestion (arrow) with large focus of sub-capsular mononuclear cell aggregation (asterisk). H. and E. X= 40.

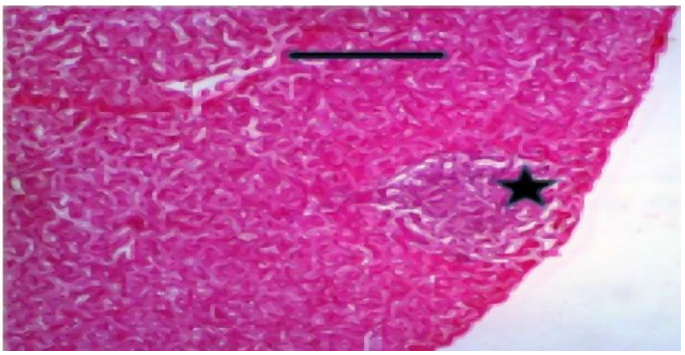

Figure 3. Liver, G3: Congestion (arrow) with large focus of sub-capsular mononuclear cell aggregation (asterisk). H. and E. X= 40.

Figure (4) shows the hepatic focus of the mononuclear aggregation (asterisk) and small sized fatty vacuolation in some of the hepatic cells (arrows).

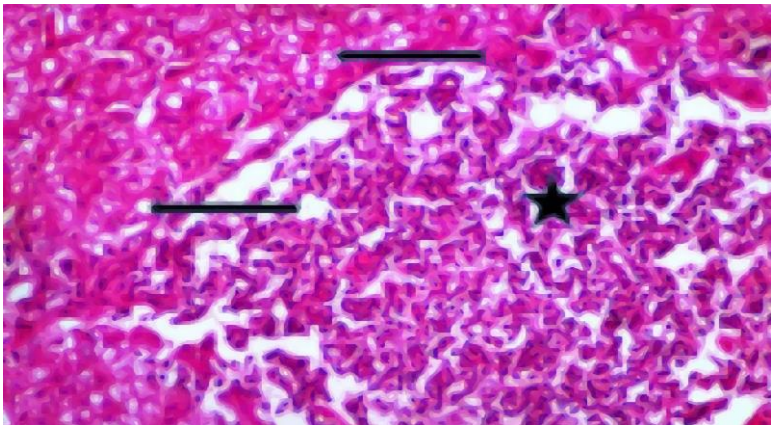

Figure 4. Liver, G3: Higher magnification of fig. 3 to show the hepatic focus of the mononuclear aggregation (asterisk) and small-sized fatty vacuolation in some of the hepatic cells (arrows). H. and E. X= 400

Table 7. Effect of dietary antibiotic, Ginger, propolis and blend of antioxidants on Growth rate(g) of Japanese quail during the period from 1 to 5 weeks of age.

Treatment mg/kg diet	Bodyweight gain (g / bird)		
	W 1-3	W3-5	W1-5
Control	125.09±1.00	53.29±1.24	152.87±0.75
Antibiotic 100 mg/kg	126.49±0.90	52.03±1.45	153.25±0.81
Ginger 125 mg/kg	125.84±0.88	50.62±1.39	152.20±0.71
Ginger 250 mg/kg	125.54±0.79	51.48±1.26	152.38±0.65
Propolis 500 mg/kg	126.75±0.73	51.60±1.11	153.23±0.70
Propolis 1000 mg/kg	126.10±0.76	52.51±1.14	153.22±0.63
Ginger 125 + Propolis 500 mg/kg	127.18±0.93	50.93±1.39	153.28±0.72
Ginger 125 + Propolis 1000 mg/kg	126.53±0.68	49.77±1.48	152.27±0.72
Ginger 250 + Propolis 500 mg/kg	125.51±0.65	51.62±1.52	152.39±0.70
Ginger 250 + Propolis1000 mg/kg	125.59±0.98	51.94±1.24	152.66±0.65
P value	**0.777**	**0.837**	**0.934**

Results illustrated in Table 5 presented the effect of dietary supplementation of antibiotic, ginger, propolis and the mixture of ginger and propolis on the growth rate of growing Japanese quail from 1 to 5 weeks of age. Results indicated insignificant effect of different supplementations on growth rate throughout the periods 1-3, 3-5 and 1-5 weeks of age.

The effects of antibiotic or propolis, ginger and their combination on hematological parameters at 21 days post-vaccination are obtained. The data revealed that RBCs and Hb were not significantly affected by different treatments, however, a significant ($P < 0.01$) increase was recorded in WBCs counts and

PCV % reached from 6.8- 54.7 % for WBCs counts and 1.2 – 29.6 % as compared with the control group. The combination of ginger plus propolis recorded the highest values of WBCs as compared to the other experimental groups.
The immunological influence of ginger and propolis was focused herein and acclimated evidence indicated that all feed additives insignificantly increased WBC's and this increase was due to greater lymphocytes production and spleen weight. The increase in lymphocytes has concurred with non-significantly greater Antibody titers against avian Newcastle disease of birds for all feed additives groups. This may reflect significant direct and indirect effects of such supplementations on the enhancement of the immunological response of the birds. Additionally, total antioxidant capacity was significantly greater with ginger and propolis and their mixture supplementation resulting in significantly less malondialdehyde than the control group, showing decreased lipid peroxidation and improved antioxidant status of birds.

Conclusion

The study investigated the effects of antibiotics, ginger *Zingiber Officinale* and propolis on the growth performance, digestibility, carcass traits, hematological indices, and serum biochemistry including the lipids profile of growing Japanese quail. Also, the present study was planned and conducted to find out the possibility of improving the immunity of growing Japanese quail through supplementing their diets with different feed additives as immunity modularity sources.
Two hundred and seventy, 7 day old unsexed growing Japanese quail chicks were divided randomly into ten groups with twenty-seven chicks in each group and each sub-group was allotted into three replicates (9 each) in a complete randomized design. Each experimental group received one of the following dietary treatments through the growing period from 1 to 5 weeks of age). In this study antibiotics (Tyrosin) and two natural feed additives, ginger and propolis and their blend were used. The order of dietary treatments was as follows:

1. Basal diet only without supplementation (served as a control).
2. Basal diet+ 100 mg antibiotic Tyrosin/kg diet (served as positive control).
3. Basal diet + 125 mg ginger /kg diet.
4. Basal diet + 250 mg ginger /kg diet.
5. Basal diet + 500 mg propolis /kg diet. (Propolis obtained from A Chinese commercial propolis as a powder from the Egyptian market (NO. HNBOOMBP-01, ISO9001 by Sea, Code: 410004900, Henan Boom, China).
6. Basal diet + 1000 mg propolis /kg diet.
7. Basal diet + 125 mg ginger +500 mg Propolis /kg diet.
8. Basal diet + 125 mg ginger +1000 mg Propolis /kg diet.
9. Basal diet + 250 mg ginger +500 mg Propolis /kg diet.
10. Basal diet + 250 mg ginger +1000 mg Propolis /kg diet.

The obtained results showed that:

1. Dietary supplementations did not significantly ($P \leq 0.05$) influence the live weight and weight gain of Japanese quail at 2, 3, 4 and 5 weeks of age.
2. The results showed significant ($P \leq 0.05$) decrease in feed intake of the groups had 250 mg ginger, 500 or 1000 mg propolis in their diet as compared with the control group through the period from 1-5 weeks age and the decrease reached to 3.0, 3.3 and 2.6 %, respectively. Also feed intake of the group had antibiotic in their diet showed significant ($P \leq 0.05$) decrease in comparison with the control group. The lowest values in feed intake were recorded in the groups given 250 mg ginger and 500 mg propolis in their diet as compared with the other experimental groups.
3. The birds received 500 mg propolis in their diet had the best ($P \leq 0.05$) record of feed conversion ratio. This group surpassed the control one by 4.2 %. The other experimental groups showed insignificant effect on this trait in comparison with the control group.
4. Most of feed additives used in the present study showed numerical increase in digestibility coefficients of most nutrients as compared to the control group. The best values were recorded in the group had 500 mg propolis in their diet. Clearly, there was insignificant improvement of 500 mg propolis supplementation / kg on digestibility coefficient of crude protein, ether extract, crude fiber, organic matter and nitrogen free extract reached to 2.9, 3.3, 92.7, 4.9 and 4.8 %, respectively, as compared to the control group. Also, the low level of ginger in the diet showed numerical increase in digestibility coefficient of crude protein, ether extract, crude fiber, organic matter and nitrogen free extract reached to 2.2, 2.5, 70.9, 3.8 and 3.7 %, respectively, in comparison with the control group. Combination of propolis and ginger powders did not give any advantage over that presented with each of propolis or ginger alone.

5. The highest value of relative carcass weight was obtained in the group that received a 500 mg propolis/kg diet. This group surpassed the control one by 3.4 % with a non-significant difference compared to the control group. However, the lowest values were recorded in the groups that had 1000 mg propolis and combinations of ginger plus propolis with non-significant differences among these groups. Antibiotic and ginger-fed groups were equal to the control group. A combination of ginger plus propolis-fed groups recorded significantly ($P \leq 0.01$) less relative carcass weight than the 500 mg propolis fed group.
6. The different supplementations did not significantly affect the absolute and relative weight of the liver, heart, gizzard, pancreas, testes, abdominal fat and rectum. However, it was observed numerically a decrease in absolute and relative weight of abdominal fat in all groups fed the different supplementations. The decrease ranged from 10.3 to 48.5 %. The lowest values in abdominal fat content were recorded in the groups fed combination of 250 mg ginger plus 500 mg propolis, 1000 mg propolis, 250 mg ginger and antibiotic, respectively, in an ascending order, respectively.
7. The absolute and relative weight of bursa of fabricious was numerically increased in birds fed diets containing 500 mg propolis, 250 mg ginger and combination of 500 mg propolis plus 250 mg ginger in an ascending order, respectively, as compared to the control and the other experimental groups. Also, most of the experimental feed additives showed a numerical increase in absolute and relative weight of thymus as compared with the control group. The increase ranged from 15.8 to 101.9 %. The absolute and relative weight of spleen showed numerical increase in the groups given different feed additives. The increase ranged from 8.3 to 52.8 % as compared with the control group.
8. The effect of different feed additives on meat quality indexes such as water holding capacity (WHC), pH, color, tenderness and also dry matter, crude protein, ether extract and ash in meat of birds were not significantly affected by different treatments as compared with the control group.
9. RBCs and Hb were not significantly affected by different treatments, however, significant ($P < 0.05$) increase was recorded in WBCs counts and PCV % reached from 6.8- 54.7 % for WBCs counts and 1.2 – 29.6 % as compared with the control group. The combination of ginger plus propolis recorded the highest values of WBCs as compared to the other experimental groups. It was observed that lymphocytes numerically increased due to the different feed additives, except in the group had combination of 125 mg ginger plus 500 mg propolis it was equal in value as the control group.
10. Antibody titters against avian Newcastle disease was non-significantly increased due to using different feed additives used in the present study compared with control group at 14 days after vaccination, except in the groups had antibiotic and combination of 250 mg ginger plus 500 mg propolis in their diet which was equal to the control group. At 21 days after vaccination, antibody titters against avian Newcastle disease were non-significantly increased only in the groups had 500 mg propolis and also in the group had combination of 250 mg ginger plus 1000 mg propolis in their diet as compared with control or the other experimental groups. Treatments with 125 mg ginger, and 250 mg ginger combined with 500 mg propolis numerically decreased antibody titters against avian Newcastle disease as compared with control group or the other experimental groups.
11. Supplementing propolis or combination of 250 mg ginger and 500 mg propolis recorded the highghst ($P \leq 0.01$) concentration values of serum total protein, followed by combination of 125 mg ginger plus 500 mg propolis and combination of 250 mg ginger plus 1000 mg propolis, respectively, as compared to the control group in a descending order. Significant ($P \leq 0.01$) differences were observed on serum albumin due to different feed additives. Propolis supplementation to the diets alone or in combination resulted in numerical increase in serum globulin as compared with control.
12. Birds given ginger alone, 500 mg propolis and combination of 250 mg ginger plus 500 mg propolis significantly ($P < 0.01$) decreased creatinine concentration as compared to the control and the other experimental groups. Uric acid concentration significantly ($P < 0.01$) decreased in the groups given 500 mg propolis and combination of 250 mg ginger plus 1000 mg propolis.
13. Supplementation of different feed additives had significant ($P < 0.05$) decreasing effect on serum AST, except the group of birds fed antibiotic in their diet showed numerical decrease in this trait. Also, ALT concentration decreased in all treated groups as compared to the control, except antibiotic and combination of 250 mg ginger plus 500 mg propolis showed non-significant numerical increase in ALT as compared with the control group. Also, Significant ($P < 0.01$) decrease in alkaline phosphatase was recorded in most of the experimental feed

additives, except in the groups had antibiotic, 250 mg ginger and the group of birds given combination of 250 mg ginger plus 500 mg propolis showed non-significant difference between them and the control group.

14. Results showed that antibiotic, ginger at 125 or 250 mg, 500 mg propolis and combination of 250 mg ginger plus 500 or 1000 mg propolis induced a significant ($P \leq 0.01$) decrease of serum total cholesterol ranged from 17.6 to 28.4 % as compared to the control group.
15. Serum total lipids and triglycerides significantly ($P \leq 0.01$) decreased with inclusion of different feed additives as compared to the control group. Non-significant decrease in concentrations of HDL was observed in the groups given antibiotic, and combination of 250 mg ginger plus 500 mg propolis as compared with the control group, however, significant ($P \leq 0.05$) increase in HDL concentration was recorded due to the other feed additives used in the present study. The group had combination of 250 mg ginger plus 1000 mg propolis in their diet showed significant ($P \leq 0.05$) increase in LDL concentration as compared with the control group.
16. Dietary treatments significantly ($P \leq 0.01$) increased total antioxidant capacity and glutathione peroxidase activity and significantly ($P \leq 0.01$) decreased malondialdehyde comparing to control group From this point of view, the results of this study indicate that growing Japanese quail fed a diet supplemented with 500 mg propolis had no significant beneficial influence on the body weights and carcass traits of chickens, but it improved feed conversion ratio, humeral immunity and was the best than antibiotic or ginger. This means that propolis might be act positively more than antibiotic and ginger under poor hygienic conditions in quail fattening. However, propolis and ginger could effectively be added to quail ration to optimize lipid profile in blood serum and diminished antioxidative status under summer heat stress conditions.

Recommendations:

Based on the results of this study, it is recommended that ginger and propolis-enriched diets be considered as a dietary supplement for Japanese quail to enhance their immune system. However, further research is required to determine the optimal dosage and duration of supplementation. Additionally, investigations into the mechanisms by which ginger and propolis exert their immunomodulatory effects would provide valuable insights.

Furthermore, it would be interesting to explore the long-term effects of ginger and propolis supplementation on the overall health, disease resistance, and productivity of Japanese quail. Comparative studies with other avian species can also be conducted to assess the potential applicability of these findings beyond Japanese quail.

In conclusion, incorporating ginger and propolis-enriched diets in the husbandry practices of Japanese quail holds promise for improving their immune system and overall well-being. The utilization of natural immunomodulators can provide a sustainable and cost-effective approach to enhance the health and disease resistance of poultry, ultimately benefiting the poultry industry and ensuring food safety for consumers.

References

[1] Akbari M, et al. (2016). Effect of dietary ginger (Zingiber officinale) extract on growth performance, antioxidant status, and immune response of broiler chickens exposed to low level of aflatoxin B1. Toxicon, 115, 109-115.

[2] Nett, J. E., & Keller, N. P. (2020). Microbial Natural Products. In Fungal Natural Products (pp. 1-23). Springer, Cham.

[3] Rober, R. Clen, Tomas, S., Banania, E. O. S., Jhone, & Herry. A. Miller, (2017). Effect of dietary ginger (Zingiber officinale) and propolis supplementation on growth, carcass traits, hematological and immunological parameters of broilers. Poultry Science, 96(9), 3201-3208.

[4] Bankova V, et al. (2014). Propolis: recent advances in chemistry and plant origin. Apidologie, 45(3), 375-392.

[5] Samadi F, et al. (2017). The immunomodulatory effects of ginger extract on the immune response of Japanese quail (Coturnix japonica). Veterinary Research Forum, 8(2), 121-126.

[6] Abdel-Moneim AM, et al. (2018). Immunomodulatory effects of propolis extract in Japanese quail challenged with Escherichia coli lipopolysaccharide. Journal of Advanced Veterinary and Animal Research, 5(3), 328-336.

[7] Gunal, M., G. Yayli, O. Kaya, N. Karahan and O. Sulak (2006). The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broiler. Int. J. Poult. Sci., 5: 149-155.

[8] Orsi, R. O., J. M. Sforcin, S. R. C. Funari and V. Bankova (2005). Effects of Brazilian and Bulgarin propolis on bactericidal activity of macrophages against Sallmonella typhimurium. Inter. Immunopharm., 5: 359-368.
[9] Niu, Z.Y.; Liu, F.Z.; Yan, Q.L.; Li, W.C. (2009). Effects of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. Poult. Sci. 88, 2101–2107.
[10] Wenk, C. (2000). Why all the discussion about herbs? Biotechnolgy in the feed industry. Proceedings of Alltech's 16th Annual Sympsium, (AAS'OO), Alltech Technical Puplications, Nottingham Universty Press, Nicholasville, KY, pp: 79-96.
[11] NRC (1994). Nutrient requirements of poultry (9th ed.). National Academy Press, Washington D.C., USA.
[12] Broody, S. (1945). Bioenergetics and growth. Reinhold Pub. W. Y., USA.
[13] Evans, J. B. and C. F. Niven (1960). Microbiology of meat; Bacteriology. In The Science of Meat and Meat Products. San Francisco: Freeman. factors associated with death loss in transit to slaughter. Can. Vet. J. 48, 76–80.
[14] Gray, R. and F. Hamm (1957). Ü̈ber das Wasserbindungsvero¨gendes Sa¨ugetiermuskels. Die Bestimmung der Wasserbindung desMuskels. Lebensmitteluntersuchung und –forschung 105: 446–460.
[15] Volovinskaia, V. P., and M. Merkolova (1958).Methods for de-termination of meat water holding capacity, office of technicalinformation, Allunion Scientific Research Institute of meat Industry, Bulletin No. 211.
[16] Yamazake, T. (1981).The effect of age and fatness on the meat quality and quantity of beef cattle. III. The changes of marbling score of the cut.
[17] A.O. A. C. (1995). official methods of Analysis 16th Edition Association of official Analytical chemists. Washington D.C.
[18] Jakobsen, P. E., K. Gertov and S. H. Nilsen (1960). Bereting fra forsogs laboratoriet, Copenhagen, 322, 56: 1-43.
[19] Hawk, P. B., B. L. Oscar and W. Summerson (1965). Hawk's [physiological chemistry]. London J., and A. Churchill Ltd. 14th Ed.
[20] Hawkey, C. M. and T. B. Dennett (1989). A color atlas of comporative veterinary hemotology. Wolf Publishing Limited, London, England.
[21] Dennit, M. Okan (2004). Effect of dietary supplementation of herb essential oils on the growth performance, carcass and intestinal characteristics of quail (Coturnix coturnix japonica). South African Journal of Animal Science 34: 79-85.