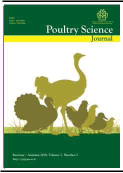

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Evaluation of the Use of Some Medicinal Plants as Diet Additive on Carcass Quality, Microbial Count and Immune Responses in Japanese Quail

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Abstract

Herbal plant supplements are one of the important natural growth promoters used as alternatives to commercial antibiotics. This experiment was conducted to investigate the effects of spice and Herbal plant products on the growth, carcass qualities, biochemical, Microbial Count and immune response factors in Japanese quail meat. A total of 360 fourteen-day-old Japanese quails female were randomly distributed into 9 groups with 4 replicates (10 quail/rep). Treatments included two levels (0.1 and 0.2%) of each herbal plant (*Trachyspermum copticum*, *Majorana hortensis*, *Stachys lavandulifolia*, *Zingiber officinale*) product and a control group that received a diet free of herbal products. From the results of this study, it was concluded that the dietary *T. copticum*, *M. hortensis* and *S. lavandulifolia* had no significant effect on live weight of Quail ($P < 0.05$). The results of this study demonstrated that the use of *Z. officinale* and *M. hortensis* increased the percentage of organs weight and *Z. officinale* increased water holding capacity, moisture ($P < 0.05$). The results of the present study revealed a significant effect of *T. copticum* herbal plant supplements on decreasing Total Bacterial Counts, Total coliform count, Triglyceride, cholesterol, pH and increasing Phosphorus and Calcium. In this experiment, *S. lavandulifolia* increased Ash and Dry matter ($P < 0.05$), while, *T. copticum*-0.2%, *Z. officinale*-0.2% and *S. lavandulifolia*-0.2% were found to increase the titres of Avian Influenza, and *M. hortensis*-0.2% increased the titres of Newcastle disease antigens. The study also demonstrated a prominent preservative effect of *T. copticum*, *S. lavandulifolia*, *Z. officinale*, *M. hortensis* and hence, *T. copticum*, *S. lavandulifolia*, *Z. officinale* and *M. hortensis* can be considered as a natural food additive in Quail and Quail products.

Introduction

The human population is projected to increase to more than 9.1 billion by the middle of this century, 34 percent higher than today. 200 million tons of annual meat production is low and must reach 470 million tons (FAO, 2009). Because of long production cycle coupled with other factors of ruminant animals such as sheep, goats, and cattle, their production hasn't been able to bridge the gap of protein stage (Muhammad *et al.*, 2017). In many countries, including Iran, poultry and pet birds are common, but in recent years, quail breeding has become common in Iran. Quail are of interest to commercial poultry breeding centers due to their rapid growth, high egg production rate, short maturity period, less space needed for breeding, resistance to most diseases and also their short incubation time (Rahmani Kahnamoei *et al.*, 2014).

The use of antibiotics as growth promoters has been prohibited by many countries as they have potential side effects on both bird and human health. Consequently, there has been a significant increase in the use of medicinal plant products in the past decade (Khan *et al.*, 2012). Among those aromatic plants, *Trachyspermum copticum*, *Majorana hortensis*, *Stachys lavandulifolia* and *Zingiber officinale* are used worldwide as spice and medicinal plants (Akbarian *et al.*, 2011; Habibi *et al.*, 2018). *Z.*

officinale is a rhizomatous herbaceous plant which contains several compounds and enzymes including gingerdiol, gingerol, gingerdione and shogaols which have antimicrobial, antioxidative and pharmacological effects (Swain *et al*., 2017). *T. copticum* and *M. hortensis* contain an important chemical called Thymol. These plants are best known as a spice and an herbal medicine for treatment and prevention of an array of diseases (Habibi *et al*., 2017). Wood betony (*S. lavandulifolia* Vahl) which is a member of the family Lamiaceae, is grown in many regions in Iran, Turkey, Iraq, Syria, Armenia as well as Georgia. (Javidnia *et al*., 2004). Alkanoids (including stachydrine and trigonelline), tannins, saponines, nicotinic acid, polyphenols, organosulfids and steroids are the main components of wood betony. Wood betony of many pharmacologically active chemical compounds which have antimicrobial activity, antioxidant activity, antifungal activity, antiviral activity, anti-inflammatory effects as well as immunomodulatory properties (Babaheydari *et al*., 2014). Herbal plant extracts act on innate immune effectors and thus modulate pathogen recognition and processing (Aroche *et al*., 2018). Medicinal plants not only are used as a growth promoter, but also induce immune system and have protective effects against many diseases (Hajiaghapour and Rezaeipour, 2018). The study of Dzinic *et al.* (2015) reported that supplementation with plant extracts in poultry diets was effective in improving overall meat quality attributes such as fillet and tender yield, sensory characteristics, organoleptic cooking parameters, overall palatability and consumer acceptability of meat (Dzinic *et al*., 2015). Several trials have been performed to evaluate the effect of Medicinal Plants on quail growth performance (Kheiri *et al*., 2018). Thus, our present trial was conducted to investigate the potential of dietary supplementation with *T. copticum*, *S. lavandulifolia*, *Z. officinale* and *M. hortensis* powder on growth, biochemical, carcass characteristics microbial count and immune responses in Japanese quail.

Materials and Methods

Experimental birds

This experimental study was carried out in Agricultural and Natural Resources College of Persian Gulf University, Iran in April 2017. A total of 360 Japanese female quails (fourteen-day-old) were used and the university ethics committee approved the study according to National Ethical Framework for Animal Research in Iran.

This experiment was conducted to investigate the effects of herbal plant products consisting of: *Trachyspermum copticum*, *Majorana hortensis*, *Stachys lavandulifolia* and *Zingiber officinale* dried powder on growth performance, carcass characteristics, biochemical, microbial count and immune responses in Japanese quail. Treatments included two levels (0.1 and 0.2%) of each herbal plant product and a control group that received a diet free of herbal products (Table 1).

Table 1. Experimental groups

Treatment	Plant name	0.1% (1 gr/1000gr)	0.2% (2 gr/1000gr)
CON	Control	-	-
MH-0.1%		+	-
MH-0.2%		-	+
ZO-0.1%		+	-
ZO-0.2%		-	+
SL-0.1%		+	-
SL-0.2%		-	+
TC-0.1%		+	-
TC-0.2%		-	+

Each treatment had four replicates, thus there was a total of 36 groups (10 quail/group). The fourteen birds with the initial weight 48.00±2.00 g was maintained and adapted under lab conditions: 16-h lighting, 8-h darkness, at temperature 25 °C±2. Feed (mash form) and water were provided *ad libitum* throughout the whole trial. The nutritional requirements of the Japanese quails were determined based on the standard requirements of the Japanese quails and the diets were formulated by the UFFDA software (Table 2) (Kheiri *et al*., 2018).

Performance and Carcass characteristics

Body weight gains per pen were evaluated at 27 and 42 days. Feed intake and feed conversion ratio were determined and calculated during each phase of the whole period. At the end of the experimental period, 5 chicks per replicate were randomly selected and sacrificed to calculate the live weight and dressed carcass weight percentages. Liver, gizzard, breast, leg, gut and abdominal fat and relative weight of each organ were calculated as follows: Relative weight = (organ weight/live body weight) ×100.

Blood parameters

Blood samples were collected from the wing veins of 8 birds/treatment (2 birds/replicate) at 42 days of age. The serum was then separated and stored at −20 °C for further analysis. Triglyceride (TG), cholesterol (cho), High density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were determined by a Shimadzu 1208 UV/VIS spectrophotometer using commercial kits Pars Azmoun (Tehran, Iran). The levels of Calcium (Ca) and Phosphorus (Pho) in serum from blood samples were measured by using commercial kits Pars Azmoun (Tehran, Iran) using autoanalyzer (Bio Systems S. A – Costa Brava 30, 08030 Barcelona Spain).

Table 2. Composition and proximate chemical analysis of basal diet

Ingredient	CON
Yellow corn%	52.56
Soybean meal (44% CP)	38.47
Gluten%	4.26
vitamin and minerals*%	0.50
Soybean oil (%)	1.45
Dicalcium phosphate (%)	0.81
Oyster shell (%)	1.56
Salt (%)	0.16
DL- Methionine (%)	0.11
L- Lysine Hydrochloride (%)	0.12
Calculated composition (%)	
Metabolizable energy, Kcal/kg	2900
Crude protein	24.00
Calcium	0.80
Available phosphorous	0.30
Sodium	0.15
Chloride	0.14
Lysine	1.30
Methionine	0.50
Methionine + Cysteine	0.88
Threonine	0.99

*Vit. A 120000 IU, Vit. D3 20000 IU, Vit. E, I00 mg, Vit. K, 20 mg, Vit. B1, 10 mg, Vit. B2, 50mg, Vit. B6, 15 mg, Vit. B12, 100 µg, Pantothenic acid 100 mg, Niacin 300 mg, Folic acid 10mg, Biotin 500 mg, Iron. 300mg, Manganese 600 mg, Choline chlorite 500 mg, Iodine 10 mg, Copper 100 mg, Selenium 1 mg, Zinc 500 mg and 1200 mg Antioxidant.

Measures of the moisture content of meat, dry matter, ash

The meat samples were dried for 24 h in an oven at 70˚C and the meat moisture was calculated as follows. The ash content was determined by charring followed by ashing the samples at 720°C to a white ash (Bouton *et al*., 1971).

Meat moisture (%) = initial weight–final weight / initial weight × 100.

Water holding capacity (WHC)

Water-holding capacity (WHC) was assessed by the method described by Jang *et al*. (2008). The breast meat of each broiler was ground in a food processor. Approximately two grams of minced meat was placed into a small polyethylene bag with small holes. The polyethylene bag with meat sample in was then fitted into a 5-mL glasses tube. The tube was centrifuged at 1500 × g for 5 mins. After centrifugation, the remained water was measured by drying the samples overnight at 70˚C (Jang *et al*., 2008).

pH Measurement

The pH of the meat samples was determined by homogenizing 10 g of meat with 50 mL of distilled water. The homogenates were filtered, and the pH of each sample was measured with a pH meter at room temperature (Bouton *et al*., 1971).

Vaccination program

All groups received Avian Influenza (AI) + Newcastle disease (ND) inactivated vaccine (subtype H9N2) via subcutaneous route at 20 days of age.

Hemagglutination inhibition (HI) test

All groups were vaccinated with AI-ND killed vaccine (subtype H9N2) subcutaneously in the breast at 20 days of age blood samples were taken on day 42 for ND antibodies and AI antibodies. Blood samples were left without anticoagulant to clot. The serum was separated by centrifugation at 1500 g for 10 min. Microtechnique of HI test was done according to Takatsy (1955). Geometric mean titer (GMT) was calculated according to Brugh (1978).

Microbiological Analysis

Samples were processed according to Ogbulie & Okpokwasili (1999). One gram of each sample breast was homogenized in 9 mL of sterile physiological saline, serial dilution carried out to 10^{-9} dilution. One mL of the solution was inoculated onto already prepared and solidified MacConkey (MAC) company Himedia and plate count agar (PCA) company Himedia using spread plate method, and incubated for 24 hours at 37°C for 24-48 hours and the colonies were counted for total plate count and the count was expressed as cfu/g (Shams Shargh *et al*., 2012).

Statistical analysis

All data were analyzed using SPSS 16.0 (SPSS Inc., USA). Normality and homogeneity of variances were tested initially using the Kolmogorov–Smirnov and Levene tests, respectively. Differences between the different groups were tested using one-way analysis of variance (ANOVA) followed by Duncan multiple comparison test. Data are presented by significant at $P < 0.05$ with means ± standard.

Results

Growth performance and carcass traits

The Growth performance and feed utilization were determined using quail live weight, feed conversion ratio and weight of carcass components (Table 3 and 4). The effect of quail meal replacement level was not statistically significant on live weight at the 27 and 42 day ($P > 0.05$). There were significant ($P < 0.05$) differences in the liver, breast, gut and gizzard among the various treatment groups throughout the experiment period.

Table 3. Effect of different dietary herbal plants ratios on Live weight (LW) and FCR

Treatment	FCR 27 to 42d	LW-27d (g)	LW-42d (g)	BWG 27-42d (g)	feed intake
CON	3.69±0.02^{de}	117.20±13.79	180.48±18.30	63.28±8.23	666.00±2.82
MH-0.1%	3.7±0.00^{d}	114.70±18.40	179.62±37.65	64.92±13.20	664.00±1.41
MH-0.2%	3.78±0.01^{b}	113.57±21.53	176.34±43.96	62.77±9.20	666.00±7.07
ZO-0.1%	3.69±0.2^{de}	116.80±13.39	180.17±26.12	63.37±10.63	664.00±5.65
ZO-0.2%	3.7±0.04^{d}	117.03±16.08	180.17±26.12	63.14±9.34	666.00±2.82
SL-0.1%	3.75±0.02^{c}	115.03±14.63	177.37±18.46	61.4±5.59	665.00±7.09
SL-0.2%	3.83±0.00^{a}	115.97±18.24	174.10±18.92	58.13±11.20	667.00±4.58
TC-0.1%	3.83±0.01^{a}	113.47±16.39	173.59±17.03	60.12±7.69	664.00±5.60
TC-0.2%	3.9±0.06	113.20±17.63	170.79±18.70	57.59±11.25	666.00±3.5
P-value	0.0001	0.71	0.91	0.57	0.11

FCR= Feed conversion ratio, LW 27d: Live weight 27 day, LW 42d: Live weight 42 day, BWG: Body weight gain.

Table 4. Effect of different dietary herbal plants ratios on Carcass traits of quail in 42 days

Treatment	Carcass traits weight (%)				
	Liver	Breast	Leg	intestine	Gizzard
CON	3.24±0.17^{abc}	32.79±0.55^{ab}	19.27±0.17	6.66±0.16^{ab}	3.54±0.30^{b}
MH-0.1%	3.61±0.44^{ab}	31.89±1.63^{ab}	19.24±2.38	7.21±0.42^{a}	4.77±0.02^{a}
MH-0.2%	3.23±0.14^{abc}	32.71±0.65^{ab}	18.67±0.42	6.11±0.74^{bc}	3.65±0.35^{b}
ZO-0.1%	2.62±0.49^{c}	33.39±1.61^{a}	17.97±1.11	5.63±0.02^{c}	3.71±0.13^{b}
ZO-0.2%	3.94±0.63^{a}	32.15±0.63^{ab}	18.88±0.29	5.69±0.09^{c}	3.70±0.14^{b}
SL-0.1%	2.87±0.31^{bc}	32.21±1.71^{ab}	17.20±1.78	5.62±1.01^{c}	3.41±0.14^{b}
SL-0.2%	3.49±0.43^{ab}	30.69±0.39^{b}	17.43±0.47	5.17±0.31^{c}	3.32±0.12^{b}
TC-0.1%	3.86±0.34^{a}	31.71±2.70^{ab}	17.66±2.20	5.93±0.23^{bc}	3.37±0.54^{b}
TC-0.2%	2.91±0.54^{bc}	31.02±0.15^{ab}	17.35±0.13	5.37±0.56^{c}	3.54±0.43^{b}
P-value	0.03	0.0001	0.27	0.0001	0.0001

$^{a-c}$ Means within a column sharing a common superscript are not different ($P < 0.05$).

Table 5. Effect of different dietary herbal plants ratios on carcass biochemical parameters of Japanese quail.

Treatment	TG (mg/dL-1)	Cho (mg/dL-1)	LDL (mg/dL-1)	HDL (mg/dL-1)	Pho (mg/dL-1)	Ca (mg/dL-1)
CON	143±26.78^{ab}	146.25±18.51^{a}	49.75±8.84	78.25±24.90	7.52±1.47^{ab}	13.42±1.17^{bc}
MH-0.1%	122±24.02^{abc}	119.33±22.14^{abc}	51.00±18.07	88.66±5.85	5.76±0.25^{bc}	12.56±0.4^{bc}
MH-0.2%	149±18.81^{a}	139.25±7.67^{ab}	49.00±8.54	80±26.26	5.40±1.55^{c}	12.42±0.59^{c}
ZO-0.1%	115.33±15.56^{bc}	121.33±28.53^{abc}	56.00±17.51	90.26±4.04	6.10±0.2^{abc}	13.53±0.73^{abc}
ZO-0.2%	131.5±7.85^{abc}	147.5±11.9^{a}	48.33±1.52	84.50±30.38	6.70±1.27^{abc}	14.50±1.01^{ab}
SL-0.1%	110±14.17^{c}	111±15.58^{bc}	59.50±18.80	91.33±11.59	6.20±0.3^{abc}	13.83±0.81^{abc}
SL-0.2%	119±9.96^{bc}	123±14.65^{abc}	52.00±5.19	80.75±28.55	6.25±1.47^{abc}	14.35±0.47^{abc}
TC-0.1%	80.66±6.80^{d}	95.33±17.55^{c}	55.50±11.26	82±9.53	7.23±0.45^{abc}	14.2±1.15^{abc}
TC-0.2%	77.25±18.2^{d}	113.5±20.37^{bc}	51.66±3.21	71.25±11.89	7.77±0.35^{a}	15.5±2.25^{a}
P-value	0.0001	0.0001	0.13	0.15	0.006	0.002

TG: Triglyceride, Cho: cholesterol, LDL: Low density lipoprotein, HDL: High density lipoprotein, Pho= Phosphorus, Ca= Calcium. $^{a-c}$ Means within a column sharing a common superscript are not different ($P < 0.05$).

Biochemical parameters

The results of different groups on TG, Cho, HDL and LDL, Ph and Ca are presented in table 5. Analysis of the obtained data showed that *T. copticum*-0.1%, *T. copticum*-0.2% and *S. lavandulifolia*-0.1% significantly decreased triglycerides and total Cho when compared to the control group. Also, groups that received *Z. officinale*-0.2% evidenced a decreased level of LDL in comparison with the control group (P < 0.05). Groups that received *T. copticum*-0.2% had increased levels of Ca and Ph in comparison with the control group (P < 0.05).

Carcass Quality

Measures of the moisture content of meat, dry matter, ash, WHC and pH characteristics of treated quail meat are given in Tables 6 and 7. We found pH, moisture, dry matter, ash, WHC did differ according to the treatment groups (P < 0.05). In this study, pH_{10d} value of the meat was determined to be between the range of 6.07-6.32, and water holding capacity of 62.43-65.46%.

Table 6. Effect of different dietary herbal plants ratios on Japanese Quails WHC, ash, moisture and dry matter on 42 days

Treatment	WHC %	Ash %	Moisture %	Dry matter %
CON	$62.90±0.45^{bcd}$	$4.36±0.15^{ab}$	$72.83±0.5^{ab}$	$27.16±0.50^{ab}$
MH-0.1%	$62.83±0.47^{d}$	$4.43±0.20^{ab}$	$73.63±0.55^{ab}$	$26.36±0.55^{ab}$
MH-0.2%	$62.43±0.80^{d}$	$4.53±0.20^{ab}$	$72.73±0.3^{b}$	$27.26±0.30^{a}$
ZO-0.1%	$65.43±1.40^{a}$	$4.56±0.15^{ab}$	$73.5±0.79^{ab}$	$26.5±0.79^{ab}$
ZO-0.2%	$65.46±1.55^{a}$	$4.43±0.20^{ab}$	$74.16±0.58^{a}$	$25.83±0.58^{b}$
SL-0.1%	$63.26±0.75^{bcd}$	$4.63±0.35^{a}$	$73.43±0.6^{ab}$	$26.56±0.60^{ab}$
SL-0.2%	$63.23±0.55^{bcd}$	$4.16±0.20^{b}$	$72.73±0.6^{b}$	$27.26±0.60^{a}$
TC-0.1%	$64.60±0.52^{abc}$	$4.2±0.10^{b}$	$73.93±0.11^{ab}$	$26.06±0.11^{ab}$
TC-0.2%	$64.26±1.17^{ab}$	$4.36±0.25^{ab}$	$73.60±1.5^{ab}$	$26.40±1.50^{ab}$
P-value	0.03	0.04	0.02	0.0001

WHC: water holding capacity. $^{a-c}$ Means within a column sharing a common superscript are not different (P < 0.05).

Table 7. Effect of different dietary herbal plants ratios on Japanese quails pH

Treatment	pH_0	pH_{1d}	pH_{3d}	pH_{10d}
CON	6.08±0.1	$5.67±0.01^{abc}$	$5.93±0.07^{d}$	$6.30±0.03^{ab}$
MH-0.1%	6.21±0.06	$5.75±0.03^{a}$	$5.99±0.10^{bcd}$	$6.29±0.09^{ab}$
MH-0.2%	6.21±0.12	$5.73±0.05^{ab}$	$5.98±0.11^{cd}$	$6.32±0.11^{a}$
ZO-0.1%	6.11±0.16	$5.56±0.03^{cd}$	$6.15±0.06^{ab}$	$6.23±0.06^{abc}$
ZO-0.2%	6±0.07	$5.51±0.06^{d}$	$6.12±0.06^{abc}$	$6.27±0.09^{abc}$
SL-0.1%	6.19±0.18	$5.68±0.09^{abc}$	$6.21±0.09^{a}$	$6.14±0.05^{cd}$
SL-0.2%	6.10±0.12	$5.65±0.06^{abc}$	$6.20±0.07^{a}$	$6.16±0.07^{bcd}$
TC-0.1%	6.19±0.08	$5.61±0.09^{bcd}$	$5.97±0.06^{cd}$	$6.13±0.08^{cd}$
TC-0.2%	6.13±0.08	$5.60±0.08^{cd}$	$6±0.10^{bcd}$	$6.07±0.05^{d}$
P-value	0.13	0.02	0.0001	0.01

$^{a-c}$ Means within a column sharing a common superscript are not different (P < 0.05).

Microbiological assessment

The number of total bacterial counts increased significantly during storage (P < 0.05). The changes in total bacterial count and total coliform count with the storage period for the treated and untreated Japanese quail are summarized in Table 8. In the present study, at the beginning of storage, the lowest value of total bacterial count (3.30 log cfu g^{-1}) was found in the group T. *copticum*-0.2% while the highest value (4.54 log cfu g^{-1}) were observed in the *Z. officinale*-0.1% and *Z. officinale* -0.2% group.

Total bacterial counts obtained in the 10 day analyses were 7.32 log cfu g^{-1}, 7.01 log cfu g^{-1}, 6.76 log cfu g^{-1}, 6.50 log cfu g^{-1}, 6.45 log cfu g^{-1}, 5.80 log cfu g^{-1}, 5.78 log cfu g^{-1}, 5.75 log cfu g^{-1} and 5.72 log cfu g^{-1} in the control, *Z. officinale*-0.1%, *Z. officinale*-0.2%, *S. lavandulifolia*-0.1%, *S. lavandulifolia*-0.2%, *T. copticum*-0.1%, *M. hortensis*-0.1%, *M. hortensis*-0.2% and *T. copticum*-0.2% groups, respectively. As shown in Table 7, the control samples had the highest Total coliform count counts. The total coliform count at storage when compared to other treatments indicated that, *T. copticum* has a strong effect against the growth of total coliform count at both concentrations of 0.1% and 0.2%. In this study, it was observed that the Japanese quail treated with *T. copticum* and *M. hortensis* supplements had lower Total coliform count levels at the beginning of storage and during storage (P < 0.05).

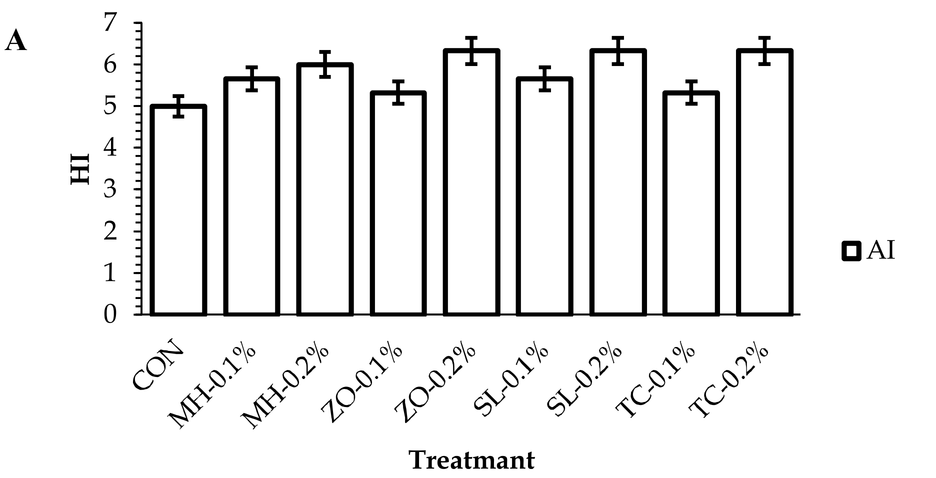
Table 8. Effect of different dietary herbal plants ratios on Total Bacterial Counts (TBC) and Total coliform count (TCC) of Japanese quails

Treatment	TBC_1	TBC_{10}	TCC_1	TCC_{10}
CON	4.40±0.23^{a}	7.32±0.2^{a}	1.96±0.69	5.1±0.55^{a}
MH-0.1%	4.01±0.72^{ab}	5.78±0.46^{cd}	1.29±1.28	4.09±0.51^{b}
MH-0.2%	4±0.61^{ab}	5.75±0.27^{d}	1.27±1.24	3.77±0.5^{b}
ZO-0.1%	4.54±0.09^{a}	7.01±0.4^{ab}	2.12±0.46	5±0.67^{a}
ZO-0.2%	4.54±0.45^{a}	6.76±0.37^{ab}	2.03±0.32	4.97±0.5^{a}
SL-0.1%	3.95±0.57^{ab}	6.50±0.48^{b}	2.11±0.8	4.16±0.53^{b}
SL-0.2%	3.87±0.24^{ab}	6.45±0.5^{bc}	2.11±0.78	3.95±0.11^{b}
TC-0.1%	3.43±0.17^{b}	5.80±0.31^{cd}	2.10±0.62	3.46±0.06^{b}
TC-0.2%	3.30±0.08^{b}	5.72±0.16^{d}	1.55±1.36	3.45±028^{b}
P-value	0.01	0.0001	0.23	0.03

TBC_1: Total Bacterial Counts on on 1 day. TBC_{10}: Total Bacterial Counts on 10 day. TCC_{10}: Total coliform count on 1 day. TCC_{10}: Total coliform count on 10 day. $^{a\text{-}c}$ Means within a column sharing a common superscript are not different ($P \leq 0.05$).

Antibody titer against ND and AI virus

The results of the Antibody titer (Avian Influenza and Newcastle disease) of Japanese quail's samples are presented in Figure 1. Using *M. hortensis*-2% in the diet significantly increases Newcastle disease in Japanese quail in comparison to both controls and different levels of other medicinal herb powders. In this study, *T. copticum*-0.2%, *Z. officinale*-0.2% and *S. lavandulifolia*-0.2% all increased the titers of Avian Influenza.



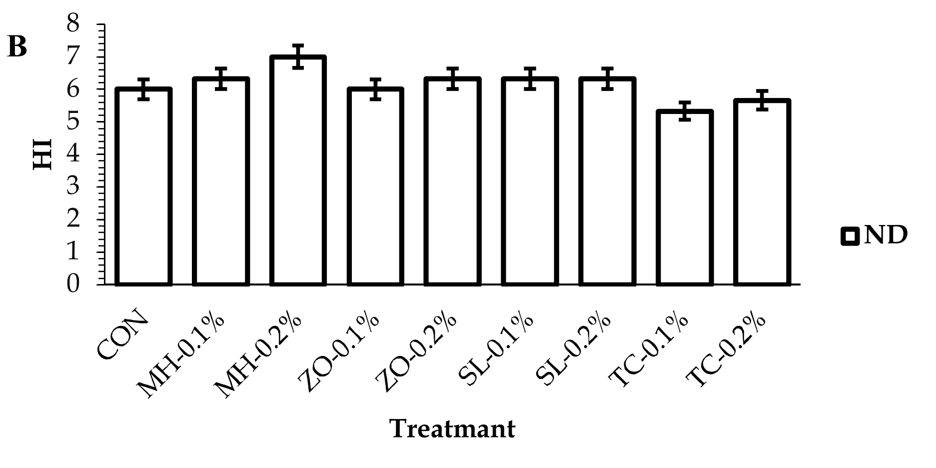


Figure 1. Effect of different dietary herbal plants ratios on antibody titer against ND and AI virus of Japanese quail ND: Newcastle disease, AI: Avian influenza. $^{a\text{-}c}$ Means within a column sharing a common superscript are not different ($P < 0.05$).

Discussion

In this study, the results of four herbal supplement products at two levels, showed no significant effect on live weight at 27 and 42 days of age and FCR compared to control group. Various properties of phenolic and flavonoid compounds of the herbal plants in the digestive system of the birds may be are the factors that change these levels. The antimicrobial and antioxidant properties of bioactive compounds of herbal plants possess that allow them to reduce the free radical and pathogenic microbe's attacks and a better use of the nutrients in the digestive tract (Herve *et al*., 2018). The results of this study demonstrated that the use of *Z. officinale* increased the percentage of breast and liver weights and *M. hortensis*-0.1% increase the percentage of leg, intestine and gizzard weight when compared with other groups. Positive effects of ginger on growth performance, gastric secretion, small intestinal morphology (villus height) and increasing digestive enzyme activities have been reported by researchers (Salmanzadeh, 2015). But some researchers reported that addition of ginger rhizome and *O. vulgare* essential oil on broiler diet had no positive effects on their live body weight (Arpášová *et al*., 2015; Saleh *et al*., 2014).

Breed, gender, age and composition of feed are the factors that affect the absence or presence of cholesterolaemic effect of essential oils in animals (Khaksar *et al*., 2012). Analysis of the obtained data showed that *T. copticum*-0.1%, *T. copticum*-0.2% and *S. lavandulifolia*-0.1% significantly decreased triglycerides and total cholesterol when compared to the control group. Also, the group receiving *Z. officinale*-2% evidence decreased levels of LDL in comparison with the control group ($P < 0.05$). The main reason for the decrease in levels of cholesterol in the quail meat can be attributable to substances such as linalool and thymol which are present in *S. lavandulifolia* and *T. copticum* (Habibi *et al*., 2018; Pirbalouti and Mohammadi, 2013).

Broilers plasma lipids and high-density lipoprotein respectively have been significantly decreased and increased by addition of different levels of *T. copticum* to broiler diets (Kheiri *et al*., 2018). In a recent experiment, Hajiaghapour and Rezaeipour (2018) reported that the supplementation of *T. copticum* essential oil decreased the serum total cholesterol, triglyceride, and glucose in Japanese quail (Hajiaghapour and Rezaeipour, 2018). Cholesterol synthesis is controlled by an enzyme named HMG-CoA (or b-hydroxy-b-methylglutaryl coenzyme A) and the inhibitory effects of thymol and carvacrol on this enzyme in chickens and subsequent reduction of Cholesterol plasma was shown in many studies (Akbarian *et al*., 2011; Kheiri *et al*., 2018). Other research reported that use of marjoram at a level of 100 mg/kg of diet is very effective on fatty oxidation and at higher levels (200 mg/kg) it delays fatty oxidation by decrease in Malonedialdehyde levels (Papageorgiou *et al*., 2003). The group receiving *T. copticum*-2% supplement evidenced an increased level of calcium and phosphorus in comparison with the control group ($P < 0.05$).

The use of nutritional strategies to improve the quality of meat is a relatively new approach that has emerged at the interface of animal and food science. Nutritional approaches are often more effective than direct addition of the additive to meat since the compound is preferably deposited where it is most needed (Dzinic *et al*., 2015). The effects of herbal plant supplementation on the qualitative factors of quail meat showed, supplementing Japanese quail diets with *M. hortensis*-0.1%, *M. hortensis*-0.2%, *Z. officinale*-0.1%, *Z. officinale*-0.2%, *S. lavandulifolia*-0.1%, *S. lavandulifolia*-0.2%, *T. copticum*-0.1% and *T. copticum*-0.2% positively influenced the WHC, Ash, Moisture and Dry matter status of Japanese quail meat. This study showed that the dietary inclusion of *Z. officinale* increased the concentrations of WHC and moisture in quail, but did not influence Ash and Dry matter. Chowdhury *et al*. (2018) reported that dietary supplementation of Herbal plants powder in chickens had resulted in appreciable differences on breast Dry matter and Total ash concentrations. Percentage of moisture in chickens did not differ due to Herbal plants powder supplementation (Chowdhury *et al*., 2018). Meat quality is closely related to the decrease in muscle pH post mortem. The pH value of meat depends on glycogen content of muscle and mechanisms of converting glycogen to lactic acid (Pethick *et al*., 1995). According to (Pearson, 1994), some processes like pH changes, should occur so that the muscle converts into meat. At the end of the postmortem process the normal pH range is between 5.6 to 6.5 (Pearson, 1994; Ranken, 2000). In this study, pH_{10d} value of the meat was determined to be between the range of 6.07-6.32.

In this study, it was observed that the Japanese quail with *T. copticum* had lower total coliform count and total bacterial count levels at the beginning of storage and during storage. Useful antimicrobial phytochemicals can be divided into several categories: phenolics and polyphenols (simple phenols, phenolic acids, quinones, flavones, tannins, and coumarins), terpenoids and essential oils, alkaloids, and lectins and polypeptides (Hajati *et al*., 2014). According to other authors, the main compounds responsible for the antimicrobial activity of *T. copticum* are thought to be thymol, β-cymene and β-pinene (Hajiaghapour and Rezaeipour, 2018). The antimicrobial action of essential oils was observed in Japanese quail that received thyme, caraway, and garlic essential oils in their diets (Behnamifar *et al*., 2015).

Nutrition is a critical determinant of immune responses. Natural products can be used as immunostimulants (Stanwell-Smith, 2001). Natural products and natural product derivatives have a traditional history as immunostimulants. The level of serum antibodies is currently an important indicator to know the effect of a new natural product on immune response in experimental animals (Aroche *et al*., 2018). In this study, *T. copticum*-0.2%, *Z. officinale*-0.2% and *S. lavandulifolia*-0.2% increased the titers of Avian Influenza, and *M. hortensis*-0.2% increased the titers of Newcastle disease. Non-specific defense mechanisms, and humoral and cellular immunities of animal immune system have been stimulated and suppressed by herbal plant supplements. Emerging evidence indicates that herbal plants exert their beneficial effects on animal immune system mostly by plant secondary metabolites (Hashemi *et al*., 2012). The authors concluded that herbal plant is an important source of antioxidants, and significantly enhances blood phagocytic activity (Karásková *et al*. 2015).

Conclusions

From the results of this study, it could be concluded that the dietary *T. copticum*, *M. hortensis*, *S. lavandulifolia* and ratio had no significant effect on live body weight at days 27 and 42 for Quail. The results of this study demonstrated that the use of *Z. officinale* increased the percentage of breast, liver, water holding capacity, Moisture and *M. hortensis*-0.1% increased the percentage of leg, gut and gizzard mass when compared with other groups. The results of the present study revealed a significant effect of *T. copticum* herbal plant supplements on decreased Total coliform count, Total Bacterial Count, Triglyceride, cholesterol, High density lipoprotein and increased Phosphorus and Calcium. In this experiment, *S. lavandulifolia* increased Ash, Dry matter and high-density lipoprotein, so, it would provide a better alternative to the commercial feed additives currently used for enhancing health and meat condition of farmed quail for human consumption.

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