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Effects of Different Levels of *Moringa oleifera* Whole Hydroalcoholic Extract and Seed Powder on the Hatching Rate, Nutritional Value, and Immune Response of Chukar Partridge Eggs

Hassan Habibi[*1], Mohammad Amin Kohanmoo[2], and Najmeh Ghahtan[3]

[1]*Department of Animal Sciences, Faculty of Agricultural and Natural Resources, Persian Gulf University, Bushehr, Iran*
[2]*Department of Plant Breeding, Faculty of Agricultural and Natural Resources, Persian Gulf University, Bushehr, Iran*
[3]*Department of Medicinal Chemistry, Faculty of Chemistry, Shiraz University of Technology, Shiraz, Iran*

*Corresponding author`s E-mail address: h.habibi@pgu.ac.ir; ORCID: 0000-0001-8162-6205

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ABSTRACT

The present study aimed to investigate the effect of different levels of *Moringa oleifera* whole seed powder (MOWSP) and whole seed hydroalcoholic extract (MOWSE) on biochemical factors including minerals, fatty acids profiles, Haugh units, cholesterol content, immune response, and hatchability rate of the eggs of Chukar partridge. A total of 225 Chukar partridge were randomly divided into five groups with three replicates of 15 birds in each group. The MOWSP was provided as a supplement at the rates of 0 g (control), 5 g, and 10 g per each kg of a diet and MOWSE at the rates of 0.5 % and 1% in drinking water. Hatchability rate and Haugh unit were, respectively, increased and decreased in all treatments in comparison with the control group. The highest and the lowest hatchability rates were recorded in the MOWSE-1% and MOWSE-0.5% supplemented groups, respectively. Birds fed with MOWSE-1% had significantly higher Iron levels than birds fed with the control diet. However, copper, zinc, and magnesium levels in the Chukar partridge eggs had no significant change, compared with the control group. Further, the C18:1, C17:0, and C16:0 of eggs were increased in response to the increase of dietary MOWSP supplementation, however, proportions of C18:0 and C18:2 decreased. It was also found that MOWSE-1% increased the antibody titers of Newcastle Disease vaccine on 69 days and MOWSP-1% and MOWSE-1% increased the titers of Avian Influenza on 59 days. It was concluded that 1% of MOWSP or MOWSE is a beneficial additive for Chukar partridge.

Keywords: *Alectoris chukar*, Cholesterol, Fatty acids profiles, Hatchability, Minerals

INTRODUCTION

By 2050, the world's population will reach 9.1 billion, which is 34% more than today. Annual meat production will need to rise by over 200 million tons to reach 470 million tons (FAO, 2009). The Chukar partridge (*Alectoris chukar*, Aves: Galliformes, hereafter, chukar) has a very wide distribution, ranging from Eurasia, China, and Mongolia in the east, to southeastern Europe in the west (Habibi et al., 2019a). The Chukar is one of the most important game birds (Barbanera et al., 2007). Chukar partridges are exploited for hunting, meat, and egg production (Pourghanbari et al., 2016). Partridge eggs are an excellent source of nutrients for humans due to having unsaturated fatty acids, low cholesterol levels, and high levels of minerals (Réhault-Godbert et al., 2019). However, there has been little research on the quality of chukar partridge eggs. Therefore, providing an effective and practical strategy to increase egg hatchability can be crucial in terms of production (Alikwe and Omotosho, 2013; Ahmad et al., 2017; Ahmad et al., 2018). Various factors can affect egg hatchability and biochemical properties such as age, genetics, gender ratio, storage period, feeding, weight of breeder animals, and egg weight (Caglayan, 2014).

Proper nutrition and the use of beneficial supplements in the diet of laying birds have been reported as main factors affecting the quality of eggs and hatchability rate (Habibi et al., 2019). In recent decades, an intensive amount of research has been focused on the development of natural growth promoters to enhance poultry production by stimulating their immune system,

reducing feed costs, and increasing weight gain. Restriction on the use of man-made antibiotic growth promoters has created the need for comprehensive research on potential alternatives. One possible alternative is phytogenic feed additives (Windisch et al., 2008). In contrast to synthetic feed additives, phytogenic additives are more favorable to customers and clear up health concerns since they are safe and eco-friendly (Imoleayo Sarah Oladeji et al., 2019). A traditional source of nutritional supplements is *Moringa* medicinal tree. There are 13 species in *Moringa* genus among which the *Moringa oleifera* (*M. oleifera)* is the most studied and long-term used species (Nur Zahirah Abd Rani et al., 2018; Abdulkarim et al., 2005; Alikwe and Omotosho, 2013). *M. oleifera* also known as the drumstick tree grows in semiarid, tropical, and subtropical areas and is used for several purposes (Worku, 2016).

M. oleifera leaf (MOL) contains 25–27% crude protein (Gadzirayi et al., 2012) and high amounts of minerals and vitamins. The protein quality of MOL has been reported to be comparable to that of milk and eggs (Castillo, 2018). Chemical analysis of *M. oleifera* seed has revealed circa ether extract, dry matter as well as ash, crude protein, crude fiber, and has been applied in animal diets (Mabruk et al., 2014, Ayasan, 2015; Ahmad et al., 2017). *M. oleifera* seeds have been reported to be a good source of proteins, minerals, and fats (Compaoré et al., 2011). Several studies have been performed to assess the effects of *M. oleifera* supplementation in broiler and layer chickens (Nkukwana et al., 2014; Mabusela et al., 2018). However, there is no literature for evaluation of the effects of *M. oleifera* supplementation on hatchability rate and egg nutritional components. Therefore, this study was conducted to investigate the effects of *M. oleifera* supplementation on hatchability rate, immune response, and egg quality.

MATERIALS AND METHODS

Experimental birds

A total of 225 (seven-month-old) chukar partridges were randomly divided into five groups with three replicates of 12 females and 3 males. The average body weight did not differ between the groups at the beginning of the trial period. All birds were allowed to adapt for a period of seven days, consuming an *ad libitum* commercial diet for laying partridge (Table 1). Strict sanitation practices were maintained in the facility throughout the experiment. The cages were cleaned daily to reduce the probability of any disease outbreak. Vaccinations and medications were imposed when deemed important during the experimental period. The control group (group T_1) was fed the same diet throughout the experiment. The remaining four groups were fed the control diet with supplementation with *M. oleifera* whole seed powder (5 and 10 g/kg for group T_2 and T_3, respectively) and 0.5% and 1% *M. oleifera* whole seed hydro-alcoholic extract in drinking water for groups T_4 and T_5, respectively.

Table 1. Composition of basal diet[1]

Ingredient	**g/kg**
Corn	518.00
Soybean meal	355.00
Soybean oil	31.40
Dicalcium phosphate	7.00
Limestone	75.00
Sodium chloride	2.80
Sodium bicarbonate	1.00
L-Lys-HCl	1.30
DL-Met	3.40
Vitamin and mineral premix[1]	5.00
Phytase 10000	0.10
Total	1000.00
Analysis	
Metabolizable energy, Kcal/kg	2800.00
Crude protein	19.84
Calcium	3.10
Available phosphorous	0.32
Sodium	0.15
Chloride	0.23
Lysine	1.08
Methionine	0.48
Methionine + Cysteine	0.88
Threonine	0.65
Tryptophan	0.22
Arginine	1.26
Isoleucine	0.77
Valine	0.83

Provided the following per kg of diet: Vitamin A, 10000 iu; Vitamin D3, 4500 IU; Vitamin E, 65 Iu; Vitamin K3, 3 mg; Vitamin B1, 2.5 mg; Vitamin B2, 6.5 mg; Vitamin B3, 60 mg; Vitamin B5, 18 mg; Vitamin B6, 3.2 mg; Vitamin biotin, 0.22 mg; Folic acid, 1.9 mg; Vitamin B12, 0.017 mg; Choline chloride, 1400 mg, Mn, 120 mg; Zn, 110 mg; Fe, 20 mg; Cu, 16 mg; I, 1.25 mg; Se, 0.3 mg

Preparation of Moringa seed powder

The seeds of *M. oleifera* were harvested from fully grown *Moringa* trees in Bushehr Province of southern Iran. Afterward, the seeds were dried, ground, and added to the diet.

Extraction of Moringa seed extract

Hydro-alcoholic extract (ethanol 70%) was prepared by seed soaking for 48 hours at room temperature and then filtered with filter paper.

Analysis of minerals

Minerals were evaluated using plasma atomic emission spectroscopy (ICP-AES, OPTIMA 5300DV, PerkinElmer, Waltham, MA) as a formerly reported procedure. In brief, 400 mg of the seed powder was weighed into a beaker and was digested in 4 mL of HNO_3-$HClO_4$ (4:1). Then it was heated to get dry. The residue then was treated with 0.1 N HNO_3 and its volume was increased to 25 mL with double-distilled water. Certified standard minerals were applied for the determination of the elements (AOAC, 1990).

Cholesterol assay

Egg collection from adult chukar was performed daily (since seven months of age). In order to measure the cholesterol, 1 g of yolks was added to 9 mL water containing 2% NaCl and was kept in a shaking rotary for 2 hours. Subsequently, 1 mL of the diluted yolk was diluted 10 times. Then, 10 µl of the sample was added to 100 µl of salt solution and 1 mL of the enzymatic reagent. Standard cholesterol also passed the same steps. Samples were kept in a water bath at 37° C for 15 minutes, and the light absorbance of the samples was measured at 500 nm wavelength. 10 µl of deionized water was used as a blank sample (Behnamifar et al., 2015).

Haugh unit

Haugh unit (HU) values were calculated using the following formula (Aboonajmi., 2010):

$$HU = 100 \text{ Log } (H + 7.57 - 1.7 \times W^{0.37})$$

Where, H is albumen height in millimeters and W denotes egg weight in gram.

Hatchability

All the experimental groups were placed into an incubator on the same date. The setter part of the incubator was set at 37.8°C and 55% RH, and eggs were automatically turned every hour. On day 20 of incubation, all the experiment eggs were transferred to a hatchery set at 37.0ºC temperatures and RH was increased to 75% and turning of eggs was stopped in all batches. At the end of the incubation period, unhatched eggs were collected and counted.

Fatty acid profile

Gas Liquid Chromatography (GLC) method was applied for the analysis of fatty acids (FA). Fatty acid methyl esters (FAME) were prepared by transesterification (Garces and Mancha, 1993), and 1 µl FAME was introduced to the GLC set and the resolution for each fatty acid was recorded. Standards of Fas were injected under the same temperature and pressure, and unknown fatty acids were detected by comparing obtained parameters with standards' ones. The levels of each FA in the FAME were measured by Shimadzu CR4-A Chromatopac. Thrombogenicity index and atherogenicity index (AI) and were assessed with the following formula:

$$AI = [(C12{:}0 + 4 \times C14{:}0 + C16{:}0)]/(\sum AGMI + \sum n-6 + \sum n-3)$$

$$TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{[(0.5 \times \sum AGMI) + 0.5 \sum n-6)} + \left(3 \times \sum n-3\right) + \sum n - \frac{3}{n} - 6)]$$

Haemagglutination inhibition test

All groups were vaccinated subcutaneously in the breast at 49 days with the killed AI-ND (H9N2 subtype) vaccine. Blood samples were taken on day 42 for ND antibodies and AI antibodies. Blood samples were left without anticoagulants to clot. The serum was dissociated by centrifugation at 3000 rpm for 10 min. Microtechnics of Haemagglutination inhibition (HI) test was performed according to Takatasy (1955). The Geometric mean titer (GMT) was calculated according to Brugh (1978).

Statistical analysis

Obtained data were analyzed by SPSS 16.0 (SPSS Inc., USA). Kolmogorov–Smirnov and Levene tests were applied to determine normality and homogeneity of the variances, respectively. Parametric data were presented as means ± standard deviation, and were compared between the dietary groups by one-way ANOVA and Duncan multiple comparison test (Duncan, 1995). The differences were considered statistically significant with $p < 0.05$.

RESULTS

Determination of minerals

The effect of *Moringa* seed extract and *Moringa* seed powder on the mean values of four minerals are shown in Table 2. Our results revealed that the iron (Fe) content of Chukar partridge eggs increased in response to the increase of dietary *Moringa* seed extract supplement ($p > 0.05$). The statistical analysis of data revealed that the

group supplemented with 1% *Moringa* seed powder recorded the highest Copper among different groups ($p > 0.05$). However, copper and magnesium levels in the Chukar partridge eggs were not significantly changed compared with the control group ($p < 0.05$).

Egg cholesterol, haugh unit, hatchability

Table 3 indicates the effect of *Moringa* seed extract and *Moringa* seed powder on the mean values of Haugh unit and cholesterol composition for different treatments. All treatment groups had lower levels of egg yolk cholesterol compared to the control group. Hatchability rate (Figure 1) and Haugh unit fractions were, respectively, increased and decreased in all treatments in comparison to the control group. The highest hatchability and the lowest value rates were recorded in the 1% *Moringa* seed extract and 0.5% *Moringa* seed powder supplemented groups, respectively.

Fatty acid profile

Fatty acid contents of total lipid in egg yolk in different treatments are presented in Table 4. The findings revealed that C18:1, C17:0, and C16:0 of Chukar partridge eggs increased in response to the increase of dietary *Moringa* seed supplementation, however, decreases were remarkable in C18:0 and C18:2.

Antibody titer against ND and AI virus

The result of the Antibody titers (Avian Influenza and Newcastle disease) of Chukar Partridge samples are presented in Figures 2 and 3. Using Moringa seed extract-1% in the diet significantly increases Newcastle disease in Japanese quail in comparison to both controls and different levels of other medicinal herb powders on 69 (Figure 2). In this study, Moringa seed powder-1% and Moringa seed extract-1% all increased the titers of Avian Influenza on the day 59 days (Figure 3).

Table 2. Mineral composition of chukar partridge eggs in different treatments (mean ± SD)

Treatment	T_1	T_2	T_3	T_4	T_5
Copper	0.654 ± 0.1^{a}	0.617 ± 0.68^{a}	0.672 ± 0.096^{a}	0.595 ± 0.098^{a}	0.585 ± 0.11^{a}
Zinc	7.75 ± 0.33^{a}	6.83 ± 0.48^{ab}	6.56 ± 1.2^{b}	7.06 ± 0.59^{ab}	7.25 ± 0.42^{ab}
Magnesium	32.13 ± 0.33^{a}	30.58 ± 1.98^{a}	30.09 ± 3.80^{a}	29.04 ± 1.24^{a}	29.99 ± 0.87^{a}
Iron	11.99 ± 0.05^{b}	12.29 ± 1.00^{b}	13.10 ± 1.76^{ab}	13.29 ± 1.45^{ab}	14.21 ± 0.60^{a}

[a-b] Means within a row sharing a common superscript are not different ($p < 0.05$). T_1: Control, T_2: 0.5% *Moringa* seed powder, T_3: 1% *Moringa* seed powder, T_4: 0.5% *Moringa* seed extract, T_5: 1% *Moringa* seed extract

Table 3. Haugh unit and cholesterol composition of chukar partridge in different treatment

Treatment	T_1	T_2	T_3	T_4	T_5
Cholesterol	22.25 ± 0.95^{a}	22.00 ± 0.81^{a}	19.25 ± 0.50^{b}	18 ± 0.81^{c}	16.25 ± 0.50^{d}
Haugh unit	79.66 ± 1.52^{a}	77.00 ± 1.73^{a}	79.00 ± 1^{a}	79.33 ± 1.52^{a}	79.00 ± 1^{a}

[a-c] Means within a row sharing a common superscript are not different ($p < 0.05$). T_1: Control, T_2: 0.5% *Moringa* seed powder, T_3: 1% *Moringa* seed powder, T_4: 0.5% *Moringa* seed extract, T_5: 1% *Moringa* seed extract

Table 4. Fatty acid composition of total lipids in egg yolk of chukar partridge in different treatments

Treatment	T_1 (%)	T_2 (%)	T_3 (%)	T_4 (%)	T_5 (%)
C14:0	0^{a}	0^{a}	0^{a}	0.07 ± 0.13^{a}	0.24 ± 0.42^{a}
C16:0	29.09 ± 3.59^{c}	30.23 ± 0.93^{bc}	30.91 ± 0.87^{bc}	32.95 ± 0.48^{ab}	34.78 ± 1.45^{a}
C16:1	0^{b}	0^{b}	0^{b}	0.17 ± 0.15^{b}	0.71 ± 0.48^{a}
C17:0	4.30 ± 0.9^{a}	4.70 ± 1.15^{a}	5.44 ± 1.64^{a}	5.63 ± 0.84^{a}	6.11 ± 1.81^{a}
C18:0	10.51 ± 2.71^{a}	8.96 ± 0.63^{ab}	6.97 ± 0.65^{bc}	5.29 ± 0.37^{c}	5.16 ± 1.05^{c}
C18:1	30.78 ± 1.65^{c}	32.84 ± 1.82^{bc}	37.37 ± 0.79^{a}	35.11 ± 1.45^{ab}	35.37 ± 0.66^{ab}
C18:2	17.00 ± 1.21^{a}	14.38 ± 1.32^{bc}	15.74 ± 2.86^{a}	14.69 ± 1.00^{a}	14.70 ± 1.63^{a}
C18:3	0^{a}	0^{a}	0^{a}	0.03 ± 0.06^{a}	0.09 ± 0.16^{a}
Unknown	2.24 ± 0.15^{b}	2.85 ± 0.33^{ab}	3.17 ± 0.22^{a}	2.96 ± 0.06^{ab}	2.91 ± 0.57^{ab}

[a-c] Means within a row sharing a common superscript are not different ($p < 0.05$). T_1: Control, T_2: 0.5% *Moringa* seed powder, T_3: 1% *Moringa* seed powder, T_4: 0.5% *Moringa* seed extract, T_5: 1% *Moringa* seed extract

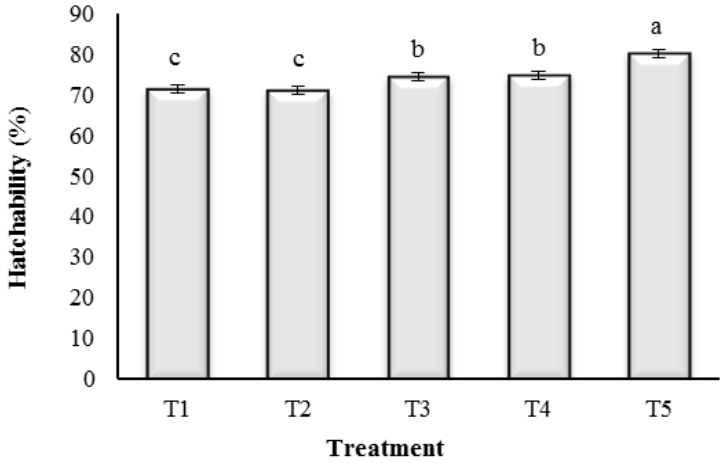
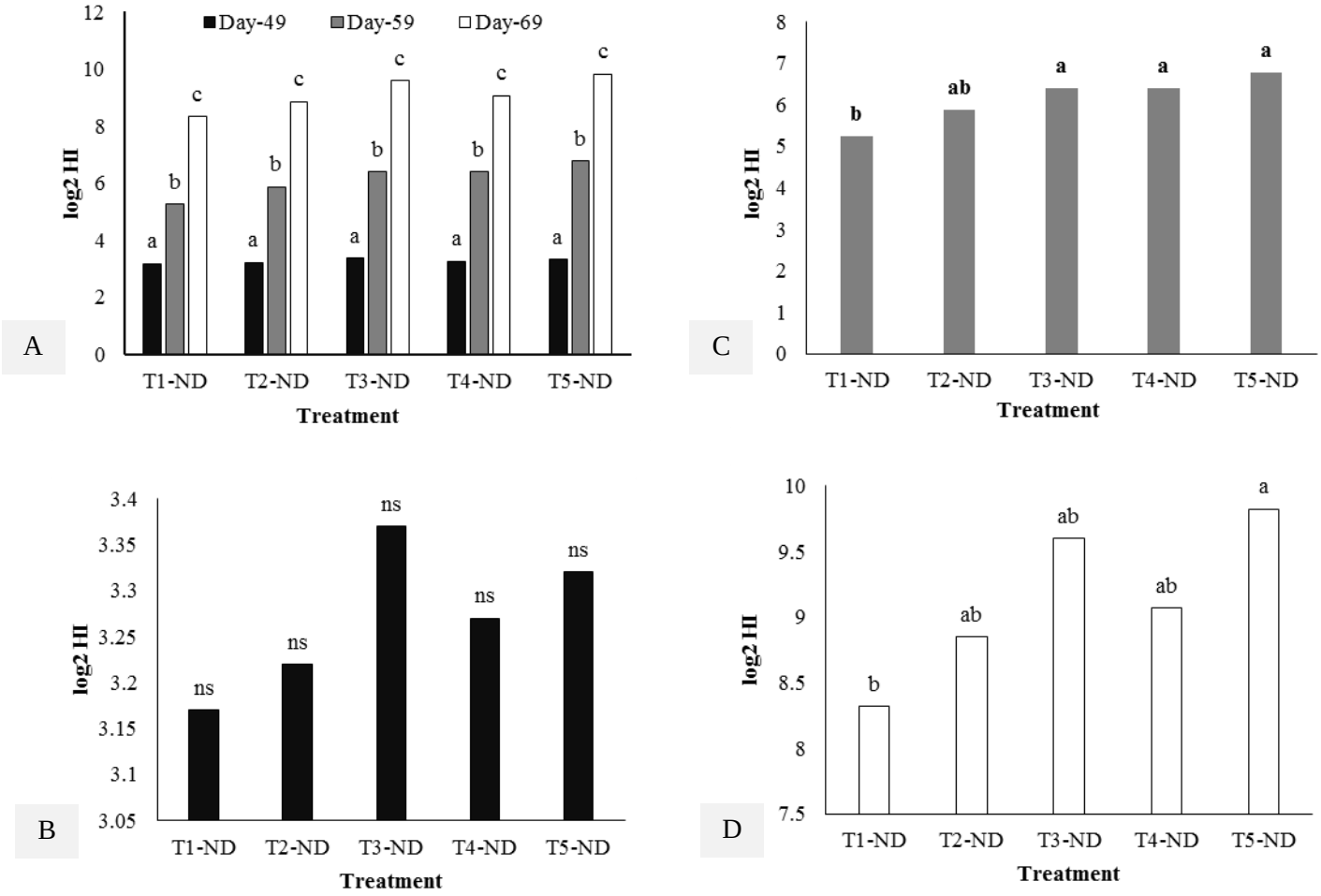
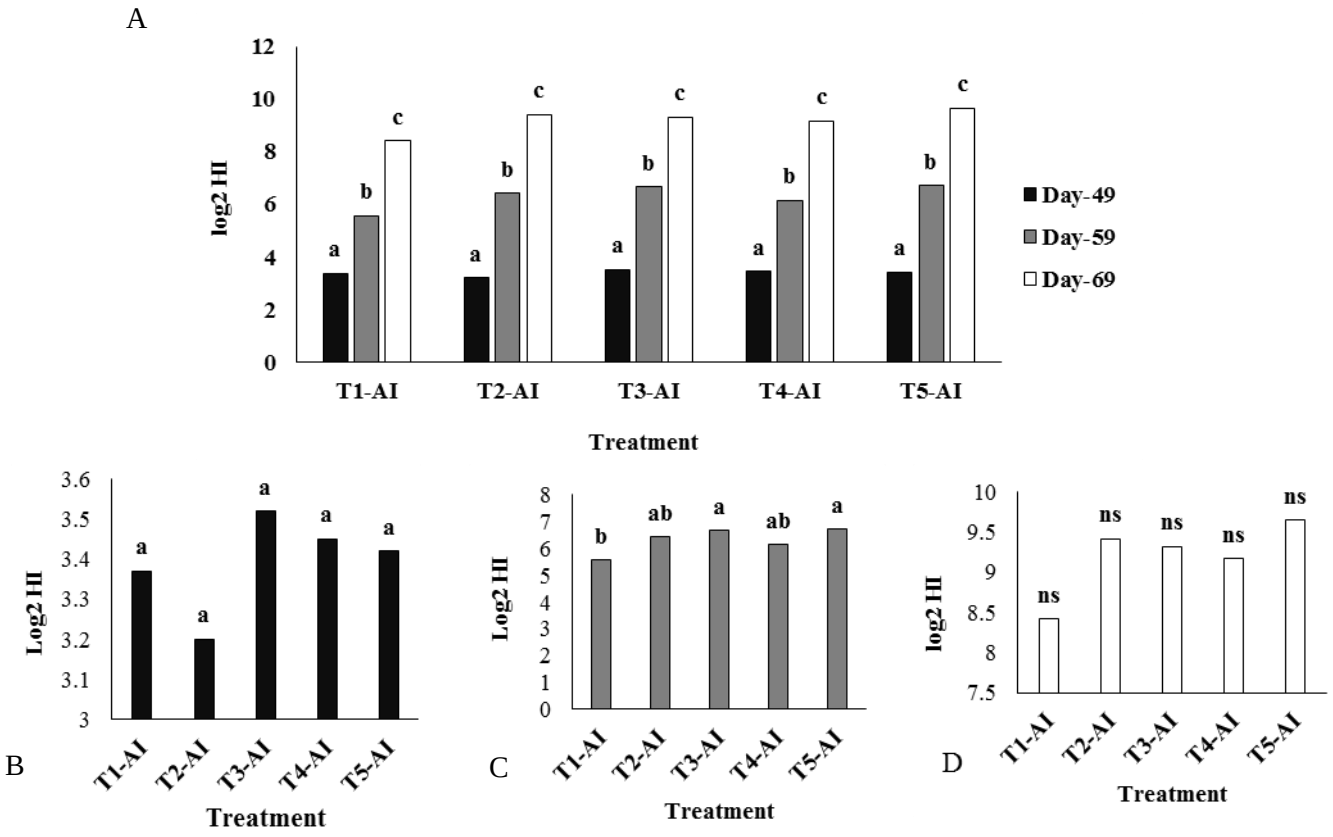


Figure 1. Hatchability of chukar partridge in different treatments. T_1: Control, T_2: 0.5% *Moringa* seed powder, T_3: 1% *Moringa* seed powder, T_4: 0.5% *Moringa* seed extract, T_5: 1% *Moringa* seed extract. The a-c above the columns show significant differences between each group ($p < 0.05$).



Figures 2. A: Effect of different dietary herbal plants ratios on antibody titer against Avian influenza (AI) virus of chukar partridge on days 49, 59, and 69. **B:** Avian influenza on day 49, **C:** Avian influenza on day 59, **D:** Avian influenza on day 69. T_1: Control, T_2: 0.5% *Moringa* seed powder, T_3: 1% *Moringa* seed powder, T_4: 0.5% *Moringa* seed extract, T_5: 1% *Moringa* seed extract. The a-c above the columns shows significant differences between each group ($p < 0.05$).



Figures 3. A: Effect of different dietary herbal plants ratios on antibody titer against Newcastle disease (AI) virus of chukar partridge on days 49, 59, and 69. **B**: Newcastle disease on day 49, **C:** Newcastle disease on day 59, **D:** Newcastle disease on day 69. T_1: Control, T_2: 0.5% *Moringa* seed powder, T_3: 1% *Moringa* seed powder, T_4: 0.5% *Moringa* seed extract, T_5: 1% *Moringa* seed extract. The a-c above the columns shows significant differences between each group ($p < 0.05$).

DISCUSSION

Herbs containing biologically active substances have been evaluated in so many studies for curative and health properties agents (Gerzilov et al., 2015) and now serve as a possible alternative nutritional supplement for optimal health, quantity, and quality of poultry products (Khan et al., 2017). *M. oleifera* has been reported to be rich in potassium, zinc, calcium, iron, and magnesium (Gopalakrishnan et al., 2016). *Moringa* powder has been also used for iron supplementation for the treatment of anemia. *Moringa* has been found to have more iron levels than spinach (Gopalakrishnan et al., 2016). Iron levels of Chukar partridge eggs were found to be the highest in the group with 1% *Moringa* seed extract ($p \geq 0.05$) and we believe that the reason is high levels of iron in *M. oleifera* (Portugaliza and Fernandez, 2012). It has been reported that phytogenic additives may significantly change the levels of minerals in poultry products (Herke et al., 2016a; Herke et al., 2016b).

Declined levels of cholesterol in the groups treated with *M. oleifera* powder might be because of a β-sitosterol because of its structural similarity to cholesterol, so, it can decrease the intestinal absorption of cholesterol (Marrufo et al., 2013; Ahmad et al., 2018). Avian embryos are supplied by lipids of the yolk, and the membrane phospholipids of many cell types in the embryo have characteristic fatty acid profiles which are related to the special functions and particular stages of tissues differentiation (Surai et al., 2001). The findings of this article showed that C18:1, C17:0, and C16:0 of Chukar partridge eggs increased in response to the increase of dietary Moringa seed supplementation, however, decreases were remarkable in C18:0 and C18:2. It has been found that a diet supplemented by *M. oleifera* can reduce the levels of short-chain fatty acids, palmitic acid, cholesterol in serum and meat, and increase the levels of unsaturated

fatty acids (UFA) in chickens (Kout Elkloub et al., 2015; Mabusela et al., 2018). Antioxidant agents such as flavonoids, ascorbic acid, and phenolics compounds could be responsible for the inhibition of cholesterol synthesis and unsaturated fatty acid could be increased by *M. oleifera* (Speake et al., 1998; Marrufo et al., 2013). There is a need for antioxidant protection in chicken embryonic tissues as they have high amounts of highly polyunsaturated fatty acids in their lipid fraction. High levels of endogenous antioxidants are critical for embryonic tissue protection during hatching as oxidative stress (Surai et al., 2016). The increase in hatchability at higher levels of *M. oleifera* seed meal can be attributed to the increase of unsaturated fatty acids (Gakuya et al., 2014; Surai et al., 2016).

Using Moringa seed extract-1% in the diet significantly increases Newcastle disease in Japanese quail in comparison to both controls and different levels of other medicinal herb powders on 69 days. In this study, Moringa seed powder-1% and Moringa seed extract-1% all increased the titers of Avian Influenza on 59 days. Non-specific defense mechanisms, and humoral and cellular immunities of the animal immune system have been stimulated and suppressed by herbal plant supplements. Nutrition is a critical determinant of immune responses. Natural products can be used as immunostimulants (Stanwell-Smith, 2001; Yassein et al., 2015). Medicinal plants having glycosides and carbohydrates are considered beneficial to immune system mechanisms by increasing body power (Yadav et al., 2014). Antimicrobial compounds (lipophilic compounds) and antioxidants (polyphenols, tannins, anthocyanins, glycosides) in *M. oleifera* may bind to the cytoplasmic membrane and destroy free radicals, activating antioxidant enzymes. As a result, it inhibits oxidases and therefore these elements are more available to birds (Jabaeen et al., 2008). Vitamins A, C and E as well as their provitamins existing in M. oleifera leaves are known to embay free radicals and may have immune protective effects (DanMalam et al., 2001).

CONCLUSION

Dietary supplementation with 0.5% or 1% *Moringa* seed powder and extract can improve cholesterol levels, hatchability, fatty acid profiles, and iron of eggs during storage, without any adverse effect on either laying performance or egg quality in chukar partridge. Therefore, it can be concluded that 1% of *Moringa* seed powder and extract are beneficial additives to the diets of chukar partridge.

DECLARATIONS

Acknowledgments

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Competing interests

The authors of this study declare no conflict of interest.

Authors' contribution

Habibi and Ghahtan were involved in the data collecting, statistical analysis, and drafting of the manuscript. Kohanmoo read and approved the final manuscript.

Ethical consideration

Ethical issues (Including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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