IMPACT OF SUPPLEMENTING DIET WITH MORINGA OLEIFERA LEAVES AND VITAMIN C ON GROWTH PERFORMANCE, BLOOD CONSTITUENTS, ANTIOXIDANT INDICES AND HORMONE PROFILES OF JAPANESE QUAILS UNDER SUBTROPICAL CLIMATIC CONDITIONS

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SUMMARY

This experiment was conducted to evaluate the Impact of supplementing diet with Moringa oleifera leaves (MOL) and vitamin C on growth performance, blood constituents, antioxidant indices and hormone profiles of Japanese quails under subtropical climatic conditions. A total 180 unsexed Japanese quails chicks, 7day old were randomly distributed into 6 groups housed with 3 replicate, 10 chicks/ each. In the 1st group (control), chicks were fed on a basal diet, while those in the 2nd (T1), 3rd (T2), 4th (T3), 5th (T4) and 6th (T5) groups were fed on the same basal diet supplemented with 3g MOL/kg diet, 6g MOL/kg diet, 200ppm Vit. C, 3g MOL/kg diet + 200ppm Vit. C, and 6g MOL/kg diet + 200ppm Vit. C respectively. Average maximum room temp°C during the day between 40-45 °C and 50% relative humidity were recorded during the experimental period until 6 weeks age. The obtained results showed that the chicks supplemented with 6g MOL/kg diet + Vit. C 200ppm had significantly greater body weight, body weight gain, Feed consumption and percentages of dressing and internal organs and improved feed conversion ratio, followed by (T2), (T4) then (T3) and (T1) compared with control group. The hematological traits, immunity parameters, antioxidant indices for chicks in treated groups were significantly ($P<0.01$) improved compared with control group. Serum proteins, albumin, globulin, high density lipoproteins (HDL) and thyroid hormones were significantly improved, while total serum lipids, cholesterol, glucose concentration, low density lipoproteins (LDL), liver enzymes, creatinine and urea were significantly ($P<0.01$) decreased compared with control group. However, no significant effects were detected on white blood cells due to the addition of different treatments compared to the control. The bacterial count in chick intestine were remarkably decreased due to addition MOL and Vit.C at different levels compared to the control. It can be concluded that MOL supplementation in Japanese quail diets is a good natural source and can be used as a safe alternative to synthetic Vit.C under subtropical climatic conditions.

Keywords: *Quail, Moringa leaves, vit. C, growth performance, subtropical climatic conditions, hormone.*

INTRODUCTION

Under tropical and subtropical regions of Egypt, some cities are affected by this climate, experiencing heat stress conditions where the peak outside ambient temperature reaches 45°C and a temperature above the critical temperature (35°C) is recorded more regularly during the summer months from June to September (Faisal, 2008). Temperature above 30°C represents a case of heat stress in poultry and is one of the most common stresses affecting production parameters. High mortality rates, low feed intake, low body weight gain, and poor feeding efficiency are effects of heat stress observed in meat-type poultry flocks (Sahin *et al.*, 2002a, b). Heat stress increases lipid oxidation as a result of increased generation of free radicals which enhances the formation of reactive oxygen species and stimulates oxidative stress in cells (Altan *et al.*, 2003). Oxidative stress demonstrated a two-fold increase in malondialdehyde as an indicator of lipid peroxidation, in skeletal muscle as a result of heat stress (Mujahid *et al.*, 2009 and Wang *et al.*, 2009) and decreased minerals and serum vitamins (Vit.) concentrations that have an important in the antioxidant defense system (Sahin *et al.*, 2002, 2009). Lipid peroxidation can be reduced by supplementation of antioxidant vitamins (Puthpongsiriporn *et al.*, 2001 and Franchini *et al.*, 2002) or by supplementation of natural substances that possess antioxidant potential (Sahin *et al.*, 2008 and Tuzcu *et al.*, 2008). V itamin C acts as an antioxidant with other antioxidants through synergistic effects. The antioxidant activity of Vit. E was increased in the presence of Vit. C by reducing tocopherol radicals to their active form of Vit. E (Doba *et al.*, 1985). Several studies have revealed that adult poultry is able to synthesize Vit. C to meet its requirements under normal conditions. However, their requirements have been found to increase during stress, and several studies have reported the beneficial effects of supplementing poultry feed with ascorbic acid (Abidin and Khatoon, 2013 and Maurice *et al.*, 2004). Dietary supplementation with Vit. C alleviated stress metabolic, improved performance, enhanced immunological status, and reduced mortality. Several studies have revealed that the optimum response in terms of growth performance, feed efficiency, feed conversion ratio (FCR), carcass quality, and survival rate in broilers under heat stress appeared with average supplements of 200-250 mg/kg Vit. C. However, the required Vit. C in laying hens is about 200–500 mg/kg to enhance feed efficiency, egg production, and egg quality under heat stress (Abidin and Khatoon, 2013, Sahin *et al.*, 2002 and Seven *et al.*, 2010). It is known that natural antioxidants such as vitamin C., flavonoids, tocopherol, and other phenolic compounds are present in some plants such as the Moringa plant. Moringa oleifera leaves are rich in Vitamin A and C, considered to be useful in catarrhal afflictions, said to have purgative properties and to promote digestion, and used as an external application for wounds (The Wealth of India1962, (A Dictionary of Indian Raw Materials and Industrial Products)).Moringa oleifera has been cultivated for decades in North Sinai, Aswan, and the New Valley. Moringa leaves are very rich in large amounts of Vit. (A, B, C, E) and carotene, polyphenols, calcium, iron, phosphorous, and protein (Murro *et al.*, 2003). Moreover, is one such plant that has been identified as containing natural antioxidants (Siddhuraju and Becker, 2003). The antioxidant effect of MOL was due to the presence of polyphenols, anthocyanins, tannins, glycosides, and thiocarbamates, which activate antioxidant enzymes, remove free radicals and inhibit oxidase (Luqmans *et al.*, 2012). Using MOL as a feed additive in poultry feeding requires an investigation of its nutritional value, as well as its effect on blood parameters as a measure of both the nutritional and medicinal benefits of the leaves in broiler chicks (Ebenebe *et al.*, 2012). Moringa is rich in nutrients that improve the digestion of other foods and reduce the activity of pathogenic bacteria and molds, helping chickens to display their natural genetic potential (Gaia, 2005). Atawodi (2010) noted that MOL contains polyphenols such as catechol, methyl gallate, ellagic acid, kaempferol quercetin, and gallate. Supplementation to the moringa diet for broilers was effective in enhancing the oxidative stability of chicken meat (Qwele *et al.*, 2013). Moringa plant, when used in poultry feed, improves growth performance and immune response under Heat stress conditions in poultry (Abou Sekken, 2015). Fouad and El-Rayes (2019) reported that supplementation of Moringa leaves to the diet improved productive performance, carcass characteristics, blood components, hormones, antioxidant indices, and immune parameters of Japanese quail chicks.

Therefore, this study was conducted to study the effect of different levels of MOL as a new source of antioxidants, and Vit. C on growth performance and physiological parameters of Japanese quail under subtropical climatic conditions.

MATERIALS AND METHODS

The experiment was carried out on a private farm in El Kharga city, New Valley Governorate, Egypt from August to September.

Moringa source and preparation:

Moringa (Moringa Oleifera) leaves were obtained from a New Valley Governorate of Egypt. The harvested moringa leaves were air-dried in the shade under a shed until they were crispy to the touch. The leaves were then milled using a hammer mill of sieve size 3 mm, to obtain a product herein referred to as moringa leaf meal (MOLM) to be ready for mixing with the other diet ingredients. The ingredients and calculated analysis of the experimental diets illustrated in Table (1a) were formulated to cover the nutrient requirements of Japanese quail recommended by the National Research Council (NRC (1994). The chemical composition of MOL is shown in Table (1b). The activities of enzymatic antioxidants and the level of non-enzymatic antioxidants in Moringa oleifera leaves are shown in (Tables 2a and 2b) according to Sreelatha and Padma (2009).

Chemical properties of vitamin C:

Ascorbic acid (AA), generally referred to as vitamin C, is a powder or crystals that are white or slightly yellow, a little acid.m.p.190°C-192°C, readily soluble in water, a little soluble in alcohol, and uncomfortably soluble in ether and chloroform and other organic solvents (LeBlanc, 2019).

Table (1a): Composition and calculated analysis of the experimental diet- through the growing period.

Ingredients	%
Ground yellow corn	53. 20
Soya bean meal (44%)	37.00
Fish meal (60.05%)	5. 50
Vegetable oil	1.00
Oyster Shel	1.00
Mono Calcium Phosphate	1.50
DL-Methionine	0.15
Salt	0.15
Minerals and vitamins premix	0.50
Calculated analysis	
Crude protein (%)	24.00
ME (kcal/kg)	2900.00
Calorie/protein ratio (C/P	120.83
Calcium (%)	1.2
Lysine	1.47
Methionine%	0. 57
Phosphorus (%)	0.55

**Each 1 kg contains: Vit. A, 12000 IU; D3, 2000 IU; E, 20 mg; K3, 3 mg; B2, 7 mg; B3, 12 mg; B5, 3 mg; B12, 0.03 mg; Biotin, 0.1 mg; Choline chloride, 300 mg; Mn, 130 mg; Fe, 70 mg; Zn, 60 mg; Cu,12 mg; I, 1 mg; Se, 0.2 mg.*

Table (1b): Chemical composition from Moringa Oleifera leaves of experimental study.

Parameter (%)	Composition%
Dry matter (DM)	94.25
Crude protein	23.80
Crude ether extract	5.50
Total ash	9.75
Nitrogen free extracts (NFE)	38.63
Crude fiber	16.57

Table (2a): Activities of enzymatic antioxidants in Moringa oleifera leaves.

Parameter	Matured leaves
SOD (Ua/g)	14.64
CAT (Ub/g)	106.84
GPx (Uc/g)	163.68
GST (U#/g)	0.30

[a] 1 Unit = Amount of enzyme that gives 50% inhibition of the extent of NBT reduction in 1 min
[b] 1 Unit = Amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units
[c] 1 Unit = Change of absorbance/minute at 430 nm d
[#] 1 Unit = µmol of CDNB conjugated/min

Table (2b): Levels of non-enzymatic antioxidants in Moringa oleifera leaves.

Parameter	Matured leaves
Ascorbic acid (mg/g)	6.60
Tocopherol (µg/g)	6.53
Total carotenoids (mg/g)	92.38

Experimental design:

One hundred and eighty, unsexed seven-day-old chicks of Japanese quails were having nearly equaled live weights and were randomly distributed into six treatment groups housed in three replicate pens (each contained 10 chicks). Chicks were raised under similar management and hygienic conditions. Feed and water were supplied ad libitum throughout the experimental period, which ended at 6 wks of age. The basal diet (control) has been formulated to meet the nutrient requirements of chicks and fed 24% CP and 2900 Kcal. The composition of the basal diet is given in Table (1a) and the levels of non-enzymatic antioxidants in MOL are recorded in Table (1b). Chicks in the first group were fed on a basal diet and considered as a control group, while the other groups 2nd (T1), 3rd (T2), 4th (T3), 5th (T4), and 6th (T5) were fed the same basal diet supplemented with 3g MOL/kg diet, 6g MOL/kg diet, Vit. C 200 ppm, 3g MOL/kg diet + Vit. C 200 ppm, and 6g MOL/kg diet + Vit. C 200 ppm respectively. Birds were individually weighed (g) at the beginning (1 week) and at the end of the experiment (6 weeks). All birds were kept under the same environmental conditions. Average maximum room temp °C during the day between 40-45 °C and 50% relative humidity were recorded during the experimental period until 6 weeks' age. Body weight (BW) and Feed consumption, BWG for each replicate, and FCR as g feed/g gain were calculated.

Temperature and humidity:

The average ambient temperature and relative humidity from August to September were recorded four times a day, twice during the day and twice at night. Average maximum room temperature between 40 °C and 45 °C during the day with 50% relative humidity (indoor) from 1 to 6 weeks of age are shown in (Tables 3).

Table (3): Average weekly room temperature and % (RH).

Month		Average temp °C during the night	Average temp °C during the day	RH %
August	Second week	27	45	50
	Third week	25	43	48
	Fourth week	26	44	49
September	Fifth week	24	43	52
	sixth week	23	40	51
Overall means		25	43	50

[1] The minimum and maximum room temperatures were recorded using digital thermometer recording minimum and maximum temperatures during the specified time (24-h period).

[2]The average values were calculated from minimum and maximum room temperature values recorded throughout the week.

Carcass and blood samples:

At 6 weeks of age, five birds from each treatment were selected randomly, weighed, and slaughtered for carcass dressing. The carcass organ weights (carcass, liver, gizzard, heart, spleen, and intestinal) were expressed as a percentage of the live weight. Total intestinal anaerobic count, total coliform count, and aerobic count (APC) were carried out according to American Public Health Association (A.P.H.A, 1985). Ten blood samples were collected from each experimental group at the time of slaughter and divided into two parts. The first part was collected in heparin tubes while the second part was collected in non-heparin tubes to obtain serum. Fresh blood aliquots were used to determine hematological parameters [red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), and white blood cells (WBCs)]. Serum was obtained from blood samples stored at 20°C for later analysis.

Measurments:

Blood biochemical parameters such as Plasma total protein (g/dl) were measured using special kits delivered from sentinel CH Milano, Italy using a spectrophotometer (Beckman DU-530, Germany) according to guidelines and recommendations of Armstrong and Carr, 1964. Plasma albumin (g/dl) was determined using special kits delivered from sentinel CH Milano, Italy according to the method of (Doumas *et al.*, 1971). Plasma globulin level (g/dl) was calculated by the difference between total protein and albumin since the fibrinogen usually comprises a negligible fraction (Sturkie, 1986). Serum total lipids concentrations (mg/dl) were determined in blood serum using special kits delivered from CAL-TECH Diagnostics, Inc., Chino, CA, the USA using a spectrophotometer according to the recommendation of (Frings *et al.*, 1972). Serum total cholesterol (mg/dl) was determined on individual bases using the specific kits according to the recommendation of (Bogin and Keller, 1987). Serum samples were analyzed for low-density lipoprotein (LDL) and high-density lipoprotein (HDL) using the colorimetric method by commercial kits obtained from Reactivos GPL, Barcelona, Spain according to the recommendation of (Warnick *et al.*, 1983). Plasma glucose concentration was measured by the method of (Trinder, 1969). The transaminase enzymes activities of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT), as U/L, were determined by the calorimetric method (Reitman and Frankel, 1957). Serum creatinine level was estimated according to (Husdan and Rapoport, 1968), while, serum uric acid level was measured according to the method explained by Patton and Crouch (1977). Plasma tri-iodothyronine (T3) and thyroxin (T4) concentrations were measured using by radioimmunoassay technique (RIA) according to (Darras *et al.*, 1991). Serum immunoglobulin (Ig) types IgG and IgM fractions were determined using the Elisa technique according to (Micini *et al.*, 1965). Total antioxidant capacity (TAC) is determined by the reaction of antioxidants in the sample with a defined amount of exogenously provided hydrogen peroxide (H2O2). The residual H2O2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5, dichloro -2- hydroxyl, (Koracevic *et al.*, 2001).

Statistical analysis:

Statistical analysis data obtained from this study were analyzed using the Statistical Analysis System (SAS, 2002) general linear model procedure, using one-way ANOVA as in the following model: $Yik = \mu + Ti + eik$ Where, Y is the dependent variable; μ is the general mean; T is the effect of experimental treatments; and e is the experimental random error. Significant differences between means were defined at $P<0.05$ compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance:

As seen from table (4), the effect of feeding Japanese quails on diets containing different levels of MOL and Vit. C on BW, BWG, FI and FCR under subtropical climatic conditions. The initial BW of quail chicks was similar for all treatments. Moringa oleifera leaves and Vit. C supplementation improved FCR and increased significantly (P<0.01) BW, BWG, and FI under subtropical climatic conditions compared with the control group. Supplementation of treatments T1, T2, T3, T4, and T5 of diet led to an increase in BW by 13.13, 18.84, 14.11, 19.58, and 27.8%, BWG by 25.1, 23.17, 17.61, 24.23 and 34.25%, FI by 5.56, 8.48, 5.86, 9.45 and 12.69%, of the control group, respectively. Moreover, quails fed the basal diet supplemented with 6g MOL/kg diet + Vit. C 200 ppm had significantly greater BW, BWG and FI followed by those fed basal diet supplemented with 3g MOL/kg diet + Vit. C 200 ppm (T4), 6g MOL/ kg diet (T2) then Vit. C 200 ppm and 3g MOL/kg diet compared to the control group.

The obtained results showed that birds fed a diet supplemented with 6g MOL/kg diet (T2) had the higher body weight, FI, BWG, and the best in FCR compared with the Vit. C 200 ppm (T4) and the control group. On the other hand, results showed that birds fed a diet supplemented with 6g MOL/kg diet + Vit. C 200 ppm (T5) had the higher FI, BW, BWG, and the best in FCR compared with the other dietary treatments and control group. It is known that natural antioxidants such as Vit.C, flavonoids, tocopherol, and other phenolic compounds are present in some plants. Moringa oleifera leaves are rich in Vit. A and C are considered to be useful in catarrhal afflictions, said to have purgative properties and to promote digestion, and are used as an external application for wounds (The Wealth of India1962, (A Dictionary of Indian Raw Materials and Industrial Products)).

Moringa leaves are very rich in large amounts of Vit. (C, E, A, B) and carotene, polyphenols, calcium, iron, phosphorous, and protein (Murro *et al.*, 2003). This improvement in the current study may be due to a reduction in bacterial load causing disease in the intestine and an improvement in the status of the intestinal lumen, resulting in increased absorption and utilization of nutrients. Moringa is rich in nutrients that improve the digestion of other foods and reduce the activity of pathogenic bacteria and molds, helping chickens to display their natural genetic potential (Gaia, 2005). Atawodi (2010) noted that MOL contains polyphenols such as catechol, methyl gallate, ellagic acid, kaempferol quercetin, and gallate. Supplementation to the moringa diet for broilers was effective in enhancing the oxidative stability of chicken meat (Qwele *et al.*, 2013). The results are in agreement with Donkor *et al.* (2013) and Teteh *et al.* (2013), who indicated that broilers fed MOL significantly increased BWG compared to broilers fed a diet without MOL. Also, the enhancement of FI of chickens fed MOL diets is consistent with the results of Melesse *et al.* (2011), who reported a higher FI for broiler chickens fed diets containing 2 and 6% MOL under subtropical climatic conditions. Melesse *et al.* (2013) found that chickens raised in tropical regions on diets containing 5% MOL consumed significantly more feed than those in the control regimen. Nkukwana *et al.* (2014) also found that BWG and FCR were the best (P<0.05) in birds supplemented with MOL (0.1-2.5%). This indicates that birds fed MOL-based diets had better nutrient utilization potential possibly due to increased size with increasing levels of inclusion (Onunkwo and George, 2015). El Tazi (2014) found that BWG, FI, and FCR rates were significantly improved (P<0.05) with the inclusion of 5% MOL in broiler feed compared to other experimental diets. On the other hand, the performance improvement with MOL groups may be due to the higher content of Vit. C in MOL, can cover the adverse effect of heat stress and enhance productive responses. El-Moniary *et al.* (2010) show that adding Vit. C to broiler feed under summer stress conditions can improve product performance. Moringa, when used in poultry feed, improves growth performance and immune response (Abou Sekken, 2015). Hassan *et al.* (2016) also found that BWG increased significantly (P<0.05) with increasing MOL level; Also, FI had the same trend. The FCR was recorded at better values with increasing MOL levels, also the addition of MOL reduces the bad effect of heat stress which helps birds to eat more feed and gain more weight and also showed that the improvement of BWG and FCR may be due to the improved CP digestibility and nutrient utilization due to the presence of flavonoids that interact as antioxidants and antibacterial or this improvement may be due to Moringa's beneficial effect on the microbial environment in the gut, which may enhance the utilization of nutrients due to improved digestion and better absorption. Fouad and El-Rayes (2019) reported that supplementation of MOL in the diet improved productive performance.

Table (4): Effect of dietary Moringa oleifera leaves (MOL) and Vit.C on growth performance of Japanese quail under subtropical climatic conditions.

Treatments/ Traits	Control	T1	T2	T3	T4	T5	SEM	P-Value
IBW (g)	35.13	34.95	35.05	34.71	34.83	34.94	0.196	0.73
BW (g)	189.67^{f}	214.59^{e}	225.41^{c}	216.45^{d}	226.82^{b}	242.40^{a}	0.191	0.01
BWG (g)	154.54^{e}	179.64^{d}	190.36^{b}	181.75^{c}	191.99^{b}	207.47^{a}	0.447	0.01
FI (g)	553.00^{d}	583.80^{c}	599.90^{b}	585.43^{c}	605.27^{b}	623.23^{a}	2.003	0.01
FCR (g feed/g gain)	3.62^{a}	3.23^{b}	3.14^{c}	3.21^{b}	3.15^{c}	3.00^{d}	0.015	0.01

[A, b, c] *Means with the different letters in the same row are significantly different (P≤0.05).*
T1= (3g MOL/ kg diet), T2= (6g MOL/ kg diet), T3= (Vit.C 200 ppm), T4= (3g MOL/ kg diet+ Vit. C 200 ppm), T5= (6g MOL/kg diet+ Vit.C 200 ppm), IBW= Initial body weight, BW= Body weight, BWG= Body weight gain, FI= Feed intake, FCR= Feed conversion ratio (g feed/g gain)

Carcass characteristics:

Data presented in Table (5) showed that the influence of dietary different sources of MOL and Vit. C supplementation on the relative weights of dressing, liver, gizzard, heart, spleen, and intestinal under subtropical climatic conditions. Results showed that the relative weight of the internal organs of Japanese quail of treated groups significantly (P<0.01) increased with Vit. C and increase of MOL level and higher than the control group. Moreover, results showed that birds fed a diet supplemented with 6g MOL/kg diet + Vit. C 200 ppm (T5) had the best dressing% compared with other dietary treatments and control groups. The improvement of dressings as a result of adding MOL supplement in the diet could be attributed to the increase in body weight at slaughter as it was suggested that a higher value of the weight of the living body is attracted and could be related to the physiological state of the high value for carcass weight (Ojewole *et al.*, 2000).

Table (5): Effect of dietary *Moringa oleifera* leaves (MOL) and Vit.C on some relative carcass characteristics of Japanese quail under subtropical climatic conditions.

Traits, %	Control	T1	T2	T3	T4	T5	SEM	P-Value
Dressing	64.46^{c}	65.94^{bc}	76.26^{a}	67.48^{b}	76.72^{a}	77.519^{a}	0.520	0.01
Liver	1.74^{e}	1.80^{d}	2.02^{c}	1.81^{d}	2.05^{b}	2.16^{a}	0.008	0.01
Gizzard	1.79^{d}	1.82^{c}	2.49^{b}	1.84^{c}	2.50^{b}	2.54^{a}	0.005	0.01
Heart	0.69^{e}	0.72^{d}	0.83^{b}	0.76^{c}	0.85^{a}	0.86^{a}	0.004	0.01

Spleen	0.040^{c}	0.045^{bc}	0.054^{a}	0.046^{b}	0.056^{a}	0.055^{a}	0.001	0.01
Intestinal	2.84^{d}	2.86^{cd}	2.95^{b}	2.88^{c}	2.98b	3.04^{a}	0.008	0.01

$^{a, b, c}$ Means with the different letters in the same row are significantly different (P≤0.05).
T1= (3g MOL/ kg diet), T2= (6g MOL/ kg diet), T3= (Vit.C 200 ppm), T4= (3g MOL/ kg diet+ Vit. C 200 ppm), T5= (6g MOL/kg diet+ Vit.C 200 ppm).

Several studies have revealed that the optimum response in terms of growth performance, feed efficiency, FCR, carcass quality, and survival rate in broilers under heat stress appeared with average supplements of 200-250 mg/kg Vit. C. (Abidin *et al.*, 2013, Sahin *et al.*, 2002 and Seven *et al.*, 2010). This important production of immune cells may also be due to the antioxidant activity of some components of MOL such as Vit. C and E (Rocha *et al.*, 2010), phenols especially flavonoids (Diallo *et al.*, 2009), and the plant's capacity of polysaccharides to modulate the immune system (Dong *et al.*, 2007). This result was also confirmed by Olugbemi *et al.* (2010) who reported improving broiler gut health with MOL. Karthivashan *et al.* (2015) reported that broiler feed 0, 0.5, 1.0, or 1.5% of MOL extract supplementation had a significantly (P<0.05) higher dressing rate compared to the control group, while 1.0% MOL showed the highest dressing rate. Kout Elkloub *et al.* (2015) reported that MOL significantly reduced abdominal fat in Japanese quail, and indicated that 0.2, 0.4, or 0.6% MOL improved spleen ratio without significant differences compared to the control group. Fouad and El-Rayes (2019) reported that adding moringa leaves to the diet improved the relative weight of chicks from the treated groups compared to the control group. In addition, birds treated with 7g MOL/kg had the best carcass weight compared to the control group and the other treatments. While Juniar *et al.* (2008) reported that the inclusion of MOL up to 10% did not produce significant effects (P<0.05) on carcass weight. Also, Ayssiwede *et al.* (2011) found that up to 24% of MOL had no effect on the organs and carcass characteristics of native Senegalese chickens. Moreover, Zanu *et al.* (2012) reported that no effect of MOL was found on all carcass traits.

Blood constituents:

Results concerning the hematological parameters of Japanese quails fed various levels of MOL and Vit. C supplementation under subtropical climatic conditions is presented in Tables (6 and 7). The results indicated that MOL and Vit. C supplementation significantly (P<0.01) increased hematological traits of quail parameters RBCs, Hb, -PCV-. However, no significant effects were detected on WBCs compared with the control. Moreover, antioxidants indices including TAC, and immune indices including IgG and IgM of quail parameters were significantly (P<0.01) increased with Vit. C and increase of MOL level were higher than the control group.

Table (6): Effect of dietary Moringa oleifera leaves (MOL) and Vit.C on some hematological blood, antioxidant indices and immune indices of Japanese quail under subtropical climatic conditions.

Traits	**Control**	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**	**P-value**
RBC(10^6/mm3)	2.78^{d}	2.89^{c}	3.63^{b}	2.92^{c}	3.66^{b}	3.76^{a}	0.009	0.01
HB(g/dl)	14.16^{d}	16.03^{c}	17.17^{bc}	17.80^{ab}	18.90^{a}	19.10^{a}	0.313	0.01
PCV%	34.83^{e}	38.27^{d}	41.00^{c}	40.23^{c}	43.47^{b}	44.97^{a}	0.438	0.01
WBC(10^3/mm3)	116.73	117.46	117.70	118.06	118.13	118.36	0.513	0.266
Lymphocytes (%)	53.10	52.83	53.80	53.36	54.30	54.10	0.484	0.278
Heterophils (%)	35.17	34.86	34.80	34.96	33.97	34.17	0.543	0.691
Eosinophils (%)	2.63	2.80	2.86	2.70	2.83	2.90	0.079	0.243
Monocytes (%)	8.53	9.50	9.10	8.97	8.90	8.83	0.486	0.842
TAC(mg/dl)	411.21^{d}	418.25^{c}	422.75^{b}	421.07^{b}	422.01^{b}	429.23^{a}	0.842	0.01
IgG (mg/100 ml)	828.53^{d}	831.33^{cd}	842.90^{b}	832.16^{c}	845.73^{b}	854.97^{a}	0.859	0.01
Igm (mg/100 ml)	228.09 ^{d}	237.30^{c}	246.37 ^{b}	239.10 ^{c}	247.97 ^{b}	257.13 ^{a}	1.008	0.01

$^{a, b, c}$ Means with the different letters in the same row are significantly different (P≤0.05).
T1= (3g MOL/ kg diet), T2= (6g MOL/ kg diet), T3= (Vit.C 200 ppm), T4= (3g MOL/ kg diet+ Vit. C 200 ppm), T5= (6g MOL/kg diet+ Vit.C 200 ppm),RBC=red blood cell; PCV=packed cell volume; WBC=white blood cell; HB= Hemoglobin TAC= total antioxidant capacity; Immunoglobulin G (IgG), IgM= Immunoglobulin M.

In addition, the results showed that birds fed a diet supplemented with 6g MOL/kg diet + Vit. C 200 ppm (T5) had the best hematological characteristics, antioxidant indices, and immune indices compared with the other treatments and the control group. Moringa oleifera leaves and Vit. C supplementation had a significant (P<0.01) improvement in blood biochemical parameters of quail such as total protein, albumin, globulin, HDL, T4, and T3 hormone while total serum lipids, cholesterol, glucose concentration, LDL, ALT, AST, creatinine and urea were significantly (P < 0.01) decreased compared to the control group. Red blood cells are responsible for manufacturing Hb, transporting oxygen and carbon dioxide in the blood, so higher values indicate better health greater potential for these functions and (Olugbemi *et al.*, 2010).

Table (7): Effect of dietary *Moringa oleifera* leaves (MOL) and Vit.C on some blood constituents of Japanese quail under subtropical climatic conditions.

Traits	Control	T1	T2	T3	T4	T5	SEM	P-value
Total protein (g/dl)	3.33[e]	3.61[d]	4.00[b]	3.64[c]	4.01[b]	4.31[a]	0.007	0.01
Albumin (g/dl)	1.40[d]	1.64[c]	1.71[b]	1.66[c]	1.71[b]	1.98[a]	0.007	0.01
Globulin (g/dl)	1.93[d]	1.97[cd]	2.29[b]	1.98[c]	2.30[ab]	2.33[a]	0.011	0.01
Glucose, (mg/dl)	270.67[a]	263.00[b]	220.33[c]	261.00[b]	218.33[cd]	216.33[d]	0.651	0.01
Total lipids (mg/dl)	294.00[a]	286.67[b]	267.33[c]	285.67[b]	267.00[c]	266.00[c]	0.788	0.01
Cholesterol(mg/dl)	265.33[a]	226.00[b]	203.66[d]	221.00[c]	201.00[d]	197.00[e]	1.011	0.01
HDL (mg/dl)	47.00[d]	56.00[c]	72.67[b]	57.67[c]	73.00[b]	88.33[a]	0.764	0.01
LDL (mg/dl)	122.33[a]	119.00[b]	112.00[c]	119.67[b]	112.33[c]	98.00[d]	0.602	0.01
AST (U/L)	58.00[a]	51.00[b]	46.00[c]	50.00[b]	45.00[c]	42.67[d]	0.628	0.01
ALT (U/L)	23.55[a]	21.72b	21.20b	21.67[b]	20.70[b]	18.37[c]	0.526	0.01
Creatinine (mg/dl)	0.197[a]	0.180[ab]	0.163[bc]	0.173[ab]	0.156[bc]	0.143[d]	0.006	0.01
Urea (mg/dl)	16.33[a]	14.00[b]	12.33[bc]	14.33[ab]	12.00[bc]	11.00[c]	0.638	0.01
T4 (ng/ml)	8.89[c]	9.37[bc]	10.77[ab]	10.67[ab]	10.90[a]	12.10[a]	0.342	0.01
T3 (ng/ml)	1.86[e]	1.97[c]	2.17[b]	2.06[c]	2.18[b]	2.33[a]	0.014	0.01

[a, b, c] Means with the different letters in the same row are significantly different (P≤0.05).
T1= (3g MOL/ kg diet), T2= (6g MOL/ kg diet), T3= (Vit.C 200 ppm), T4= (3g MOL/ kg diet+ Vit. C 200 ppm), T5= (6g MOL/kg diet+ Vit.C 200 ppm), AST=aspartate amino transferase; ALT=alanine amino transferase; HDL=high-density lipoprotein; LDL=low-density lipoprotein; T3= triiodothyronine; T4=thyroxine;

Moringa oleifera leaves have a beneficial effect on immune responses (Olugbemi *et al.*, 2010). These results were in agreement with Onu and Aniebo (2011), which found that PCV was significantly higher in birds fed 2.5 or 5% MOL compared to control birds. Sameh (2017) showed that chicks fed 5% MOL had significantly higher PCV than the control group and those supplemented with 8% MOL, while birds fed 3% MOL had average medium and no difference from others. Makanjuola *et al.* (2014) noted that the decrease in ALT activity observed in birds on the MOL diet could indicate that it has properties that can promote liver health. In addition, it has been used as a natural antioxidant due to its antioxidant activity and is rich in antioxidant compounds such as ascorbic acid, flavonoids, carotenoids, and phenols (Vongsak *et al.*, 2014). Moringa oleifera extract (in both the mature and tender leaves have strong antioxidant activity against free radicals, provides significant protection against oxidative stress, and prevents oxidative damage to main biomolecules (Sreelatha and Padma, 2009). Kout Elkloub *et al.* (2015) reported that MOL significantly reduced plasma cholesterol, especially LDL, and improved the immune system and blood components in Japanese quail. Hassan *et al.* (2016) reported that MOL addition up to 0.3% improved physiological parameters and enhanced the ability to resist heat stress conditions for broilers where Hb increased with increasing MOL level, while hematocrit (Ht) values did not affect. Plasma total protein, globulin, and thyroid hormones (T3 and T4) increased significantly (P<0.05) with increasing MOL levels. Albumin was not affected. While the AST was significantly decreased (P<0.05). Fouad and El-Rayes (2019) reported that supplementation of Moringa leaves at levels of 3, 5, and 7g MOL/kg to the diet improved blood components, hormones, antioxidant indices, and immune parameters.

Bacteriological count:

The results of the intestinal microbial count of Japanese quail are presented in Table (8). All levels of MOL and Vit. C supplementation under subtropical climatic conditions led to a decrease in the total aerobic count, total coliform, and total anaerobic count, compared with the control group. In this study, the beneficial effect on the number of gut microbes may be due to the addition of MOL to bird feed because it contains flavonoid and phenol components, as well as its antimicrobial activity (Luqmans *et al.*, 2012). Moreover, MOL is rich in nutrients that improve the digestion of other foods and reduce the activity of pathogenic bacteria and molds, helping chickens to display their natural genetic potential (Gaia, 2005). Atawodi (2010) noted that MOL contains polyphenols such as catechol, methyl gallate, ellagic acid, kaempferol quercetin, and gallate. Supplementation to the MOL diet for broilers was effective in enhancing the oxidative stability of chicken meat (Qwele *et al.*, 2013).

Table (8): Effect of dietary *Moringa oleifera* leaves (MOL) and Vit.C on total anaerobic, total coliform and aerobic count of bacteria in intestine of Japanese quail under subtropical climatic conditions.

Traits	Control	T1	T2	T3	T4	T5	SEM	P-value
Total anaerobic count x10^3	0.97^a	0.80^{ab}	0.40^c	0.77^b	0.37^c	0.30^c	0.056	0.01
Total coliform Count x10^3	11.00^a	9.00^b	6.00^c	9.33^{ab}	5.50^c	4.80^c	0.593	0.01
Aerobic count x10^3	7.80^a	5.53^b	4.13^{cd}	5.30^{bc}	3.87^d	1.67^e	0.393	0.01

[A, b, c] Means with the different letters in the same row are significantly different (P≤0.05).
T1= (3g MOL/ kg diet), T2= (6g MOL/ kg diet), T3= (Vit.C 200 ppm), T4= (3g MOL/ kg diet+ Vit. C 200 ppm), T5= (6g MOL/kg diet+ Vit.C 200 ppm).

CONCLUSION

It can be concluded that MOL supplementation in Japanese quail diets is a good natural source and can be used as a safe alternative to synthetic Vit.C under subtropical climatic conditions.

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تأثير اضافة أوراق المورينجا أوليفيرا وفيتامين ج على أداء النمو ومكونات الدم ومضادات الأكسدة والخصائص الهرمونية لطائر السمان الياباني تحت الظروف المناخية شبه الاستوائية

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أجريت هذه التجربة لتقييم تأثير أوراق المورينجا (MOL) كمصدر طبيعي آمن في علائق السمان كبديل لفيتامين ج المخلق صناعياً تحت الظروف المناخية شبه الاستوائية على الأداء الإنتاجي ومكونات الدم والمناعة والخصائص المضادة للأكسدة والهرمونات وخصائص الذبيحة وعدد البكتيريا في الأمعاء. اشتملت هذه الدراسة على عدد 180 كتكوتً سمان ياباني عمر سبعة أيام قسمت عشوائياً على 6 معاملات تجريبية بكل منها 3 مكررات بكل مكرر 10 كتاكيت. تمت تغذية كتاكيت المجموعة الأولى على العليقة الاساسية بدون أي إضافة (كنترول) ، بينما تمت غذيت المجموعات الأخرى الثانية (T1) ، والثالثة (T2) ، والرابعة (T3) ، والخامسة (T4) ، والسادسة (T5) على العليقة الاساسية مضاف إليها 3 جم من ورق المورينجا (MOL) / كجم علف ، 6 جم (MOL)/ كجم علف ، فيتامين C (200 جزء في المليون) ، 3 جم (MOL)/ كجم علف + فيتامين C (200 جزء في المليون)، و 6 جم (MOL)/ كجم علف + فيتامين C (200 جزء في المليون)على التوالي ، وتراوح متوسط درجة الحرارة بين 40 درجة مئوية و 45 درجة مئوية مع رطوبة نسبية 50٪ خلال فترة التجربة حتى عمر 6 أسابيع.

أظهرت النتائج ان اضافة 6 جم (MOL)/ كجم علف + فيتامين C (200 جزء في المليون) ادى الى زيادة معنوية فى وزن الجسم الحي ومعدل الزيادة فى وزن الجسم والعلف المستهلك مع تحسن الكفاءة الغذائية ونسبة الذبيحة، بالضافى الى تحسن نسب الاعضاء الداخلية للسمان اليابانى تليها فى ذلك المعامله (T2)و (T4) ثم (T3) و (T1) مقارنة بمجموعه الكنترول. كان للمعاملة زيادة ملحوظة (P<0.01)في مكونات الدم والمناعة والخصائص المضادة للأكسدة، وتحسين صفات الدم الكيميائية مثل البروتين الكلي، والألبومين، والجلوبيولين، والبروتينات الدهنية عالية الكثافة، وهرمونات الغدة الدرقية بالمقارنة بالكنترول وانخفضت نسبة الدهون الكلية والكوليسترول وتركيز الجلوكوز والبروتينات الدهنية منخفضة الكثافة LDL وأنزيمات الكبد والكرياتينين واليوريا معنويا (P<0.01) مقارنة بمجموعة الكنترول وكذلك لم توجد أي فروق معنوية في خلايا الدم البيضاء بين المعاملات والكنترول. انخفض عدد البكتيريا في الامعاء نتيجة إضافة المعاملات المختلفة مقارنة بمجموعة الكنترول. لذلك يمكن الاستنتاج أنه يمكن اضافة أوراق المورينجا (MOL) لعلف السمان الياباني كمصدر طبيعي جيدة وبديل آمن بدلاً من فيتامين Cالمخلق صناعيا تحت الظروف المناخية شبه الاستوائية.

مفاتيح البحث: *السمان، أوراق المورينجا، فيتامين ج، أداء النمو، الظروف المناخية شبه الاستوائية، الهرمون.*