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

Certain cities in Egypt's tropical and subtropical regions are subject to heat stress conditions, with highs outside ambient temperature reaching 45°C and more frequent records of temperatures above the critical point of 35°C during the summer months of June through September (Faisal, 2008). One of the most frequent pressures impacting production parameters is temperature, and in poultry, a temperature above 30°C is indicative of heat stress. Heat stress in meat-type poultry flocks has been linked to high mortality rates, low feed intake, low body weight increase, and poor feeding efficiency (Sahin et al., 2002a, b). Heat stress causes a rise in free radical production, which in turn promotes the production of reactive oxygen species and oxidative stress, increasing lipid oxidation (Altan et al., 2003). As a result of heat stress, oxidative stress showed a two-fold increase in malondialdehyde, an indicator of lipid peroxidation, in skeletal muscle (Mujahid et al., 2009 and Wang et al., 2009). It also showed decreased concentrations of minerals and serum vitamins (Vit.), which are crucial for the antioxidant defense system (Sahin et al., 2002, 2009). Antioxidant vitamin supplements (Puthpongsiriporn et al., 2001; Franchini et al., 2002) or natural substances with antioxidant potential (Sahin et al., 2008 and Tuzcu et al., 2008) can both lower lipid peroxidation.

Vitamin 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 Japanese quail chicks, aged seven days, were randomly assigned to six treatment groups,each groups housed in three replicate pens each consisting of ten chicks. The chicks' live weights were almost similar. Under comparable supervision and hygienic settings, chicks were grown. Water and food were given freely during the trial, which concluded after six weeks of age. In order to satisfy the nutritional needs of chicks, the basal diet (control) is designed to provide 24% CP and 2900 Kcal. Table (1a) lists the components of the basal diet, and Table (1b) lists the concentrations of non-enzymatic antioxidants in MOL. The first group of chicks was fed a basal diet and was regarded as the control group. The other groups, which were T1, T2, T3, T4, T5, and T6, were fed the same basal diet supplemented with 3g MOL/kg diet, 6g MOL/kg diet, and 200 ppm of vitamin C, as well as 3g MOL/kg diet + Vit. C 200 ppm, and 6g MOL/kg diet + Vit. C 200 ppm , respectively. Each bird was weighed in grams both at the start of the experiment (1 week) and at the conclusion (6 weeks). Every bird was housed under identical environmental circumstances. Over the course of the trial, up until six weeks of age, the average highest room temperature ̧C during the day was reported between 40 and 45 °C with 50% relative humidity. Body weight (BW) and Feed consumption, BWG for each replicate, and FCR as g feed/g gain were calculated.

Temperature and humidity:

Between August and September, four daily readings of mean the ambient temperature and relative humidity were made twice in the day and twice at night. From one to six weeks of age, the average maximum room temperature, measured indoors, is between 40 and 45 degrees Celsius during the day with 50% relative humidity (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:

Five birds from each treatment were chosen at random, weighed, and killed for carcass dressing when they were six weeks old. The percentage of the live weight was used to express the weights of the carcass, liver, gizzard, heart, spleen, and intestinal organs. The American Public Health Association (A.P.H.A, 1985) was followed in the execution of the total intestinal anaerobic count, total coliform count, and aerobic count (APC). At the time of slaughter, ten blood samples from each experimental group were taken and split into two sections. To get serum, the first half was collected in heparin-containing tubes, and the second part was taken in non-heparin-containing tubes. Hematological parameters (red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), and white blood cells (WBCs)) were measured using fresh blood aliquots.

Measurements:

In accordance with the principles and procedures of Armstrong and Carr, 1964, blood biochemical parameters such as plasma total protein (g/dl) were determined using specialized kits supplied by sentinel CH Milano, Italy using a spectrophotometer (Beckman DU-530, Germany). Using specialized kits sent from sentinel CH Milano, Italy, plasma albumin (g/dl) was measured using the protocol of (Doumas et al., 1971). Since fibrinogen often consists of a small fraction, the plasma globulin level (g/dl) was determined by calculating the difference between total protein and albumin (Sturkie, 1986). Following the suggestion of Frings et al. (1972), serum total lipid concentrations (mg/dl) in blood serum were measured using spectrophotometer and specific kits supplied by CAL-TECH Diagnostics, Inc., Chino, CA, USA. 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:

The general linear model process of the Statistical Analysis System (SAS, 2002) was used to examine the statistical analysis data. One-way ANOVA was used in this model: Yik= µ+ 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:

Table (4) illustrates the impact of feeding Japanese quails diets with varying MOL and Vit. C concentrations on BW, BWG, FI, and FCR in subtropical climates. For every treatment, the quail chicks' initial BW was the same. Under subtropical climate circumstances, supplementing with vitamin C and Moringa oleifera leaves improved FCR and significantly (P<0.01) raised BW, BWG, and FI in comparison to the control group. When diet treatments T1, T2, T3, T4, and T5 were added, the BW, BWG, and FI of the control group increased by 13.13, 18.84, 14.11, 19.58, and 27.8%, 25.1, 23.17, 17.61, 24.23, and 34.25%, and 5.56, 8.48, 5.86, 9.45, and 12.69%, respectively. Furthermore, in comparison to the control group, quails fed the basal diet supplemented with 6g MOL/kg diet + Vit. C 200 ppm(T5), 3g MOL/kg diet + Vit. C 200 ppm (T4), 6g MOL/kg diet (T2), then Vit. C 200 ppm and 3g MOL/kg diet had significantly higher BW, BWG, and FI.

When compared the Vit. C 200 ppm group (T3) and the control group with 6g MOL/kg food (T2), the results indicated that the birds fed a diet supplemented with 6g MOL/kg food (T2) had the highest body weight, FI, BWG, and the best FCR. However, when compared to the other dietary treatments and the control group, the results indicated that the birds fed a diet supplemented with 6g MOL/kg food + Vit. C 200 ppm (T5) had the highest FI, BW, BWG, and the best FCR. It is well recognized that several plants contain naturally occurring antioxidants such vitamin C, flavonoids, tocopherol, and other phenolic substances. Rich in vitamins A and C, Moringa oleifera leaves are used externally for wound healing, believed to have purgative qualities, aid in digestion, and be beneficial in catarrhal ailments (The Wealth of India, 1962, A Dictionary of Indian Raw Materials and Industrial Products).

Large concentrations of vitamin C, E, A, and B, carotene, polyphenols, calcium, iron, phosphorus, and protein can all be found in moringa leaves (Murro et al., 2003). The present study suggests that the observed improvement could be attributed to a decrease in the amount of bacteria causing intestinal disease and an enhancement in the intestinal lumen's condition, leading to better nutritional absorption and utilization. Rich in nutrients, moringa helps hens reach their full genetic potential by enhancing their ability to digest other foods and suppressing the growth of mildew and harmful bacteria (Gaia, 2005). Polyphenols including catechol, methyl gallate, ellagic acid, kaempferol, quercetin, and gallate are found in MOL, according to Atawodi (2010). The oxidative stability of chicken meat was successfully increased by adding supplements to the broiler diet that contained moringa (Qwele et al., 2013). The findings concur with those of Donkor et al. (2013) and Teteh et al. (2013), who reported that broilers given MOL had a considerably higher BWG than broilers fed a diet without MOL. Furthermore, the increase in FI of chickens fed MOL diets agrees with the findings of Melesse et al. (2011), who noted a greater FI for broiler chickens fed diets.

Additionally, BWG and FCR were highest (P<0.05) in birds supplemented with MOL (0.1-2.5%), according to Nkukwana et al. (2014). This suggests that birds fed meals based on MOL had improved capability for using nutrients, presumably as a result of becoming larger as inclusion levels increased (Onunkwo and George, 2015). El Tazi (2014) discovered that adding 5% MOL to broiler feed considerably (P<0.05) increased the rates of BWG, FI, and FCR when compared to other experimental diets. However, it's possible that the MOL groups' improved performance was brought about by the vitamin C concentration of MOL, which might mitigate the negative effects of heat stress and promote positive reactions. According to El-Moniary et al. (2010), broiler feed supplemented with vitamin C during summer stress conditions can enhance product performance. When added to poultry feed, moringa enhances immunological response and growth performance (Abou Sekken, 2015). According to Hassan et al. (2016), FI followed the same trend as BWG, increasing considerably (P<0.05) as the MOL level rose. As MOL levels rose, the FCR was reported at better values. Additionally, the addition of MOL lessens the negative effects of heat stress, allowing birds to consume more feed and gain weight. It also demonstrated that the improvement in BWG and FCR could be attributed to either improved CP digestibility or nutrient utilization as a result of flavonoids acting as antioxidants and antibacterials, or to the positive effects of Moringa on the gut microbial environment, which could improve nutrient utilization through better absorption and digestion. According to research by Fouad and El-Rayes (2019), adding MOL to the diet enhanced 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:

Under subtropical climate circumstances, the data given in Table (5) demonstrated the impact of dietary sources of MOL and vitamin C supplementation on the relative weights of dressing, liver, gizzard, heart, spleen, and intestinal. The internal organ relative weight of the treated groups of Japanese quail grew significantly (P<0.01) increased with vitamin C and an increase in MOL level, and was greater than that of the control group, according to the results. Furthermore, when compared to other dietary treatments and control groups, the results indicated that birds fed a diet supplemented with 6g MOL/kg food + Vit. C 200 ppm (T5) had the best dressing percentage. The rise in body weight at slaughter may be related to the improvement in dressings brought about by including MOL supplement in the meal.The improvement in dressings as a result of adding MOL supplements in the diet could be attributed to the increase in body weight at slaughter as it has been suggested that higher value of live body weight is attracted and could be related to the physiological condition of high value of 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:

Tables 6 and 7 report the results of the hematological parameters of Japanese quails fed different doses of MOL and vitamin C supplementation in subtropical climate conditions. The findings showed that quail metrics RBCs, Hb, and -PCV-hematological characteristics were considerably (P<0.01) elevated by MOL and vitamin C supplementation. On WBCs, however, no discernible changes were found as compared to the control. Furthermore, there was a substantial (P<0.01) increase in antioxidant indices like TAC and immunological indices like IgG and IgM of quail parameters with vitamin C, and the MOL level increased more than that of 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.

Furthermore, the findings demonstrated that, in comparison to the other treatments and the control group, birds fed a diet supplemented with 6g MOL/kg food + Vit. C 200 ppm (T5) had the best hematological features, antioxidant indices, and immunological indices. The blood biochemical parameters of quail, including total protein, albumin, globulin, HDL, T4, and T3 hormone, significantly improved (P<0.01) when Moringa oleifera leaves and vitamin C supplementation were given.

Conversely, total serum lipids, cholesterol, glucose concentration, LDL, ALT, AST, creatinine, and urea significantly decreased (P<0.01) when compared to the control group. Higher numbers suggest better health and greater capacity for these functions since red blood cells are important for producing hemoglobin and transferring oxygen and carbon dioxide in the blood (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.*