EFFECT OF SPRAYING MORINGA OIL ON EMBRYONIC DEVELOPMENT, HATCHABILITY, PHYSIOLOGICAL PARAMETERS, POST-HATCH CHICK GROWTH AND BACTERIAL CONTAMINATION OF FERTILE QUAIL EGGS

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SUMMARY

Moringa oil has strong antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria in addition to anti-fungal properties. This study evaluated the impact of spraying hatching eggs of Japanese quail with Moringa oil solution on embryonic development, physiological parameters, hatchability, post-hatch chick growth, and bacterial load on the surface of the eggshell. Seven hundred and fifty quail eggs were divided equally into 5 experimental groups. Eggs in the 1st group were presented as a control (untreated eggs), while, those in the 2nd group served as control but were only sprayed with an organic solvent 70% ethyl alcohol. Eggs of the 3rd, 4th and 5th groups were sprayed with 2, 4 and 6 ml Moringa oil solution /liter, respectively. Embryo weight, body length, shank length, chick weight, chick body length, chick shank length, and hatchability tended to be significantly higher ($P<0.05$) in eggs treated with Moringa oil solution compared with control groups. While embryonic mortality, hatch time, egg albumen weight ratio, eggshell thickness, and egg weight loss ratio at 14 days of incubation were significantly decreased ($P<0.05$) in the eggs that were sprayed with the Moringa oil solution compared to control eggs. The means of RBCs, hemoglobin, PCV, total protein, albumin, globulin, T4 hormone and IgG were significantly increased ($P<0.05$) at 1 and 14 days of age. The white blood cell count was slightly increased in response to the Moringa oil solution. The body weight, body weight gain, and feed intake of chicks at 14 days of age recorded high significant values in response to spraying with Moringa oil solution. In contrast, the percentage of yolk residual at hatch was lower than that of the control group. Treating Moringa oil solution had a significant effect on TBC and coliforms on the eggshell surface after 24 h, 7, and 14 days of incubation compared to control groups. The number of total aerobic and anaerobic bacteria in the chicks and the total coliform bacteria count decreased. In conclusion, spraying Japanese quail eggs with a solution of Moringa oil as a natural disinfectant (pre-incubation) is a good way to improve embryonic development, hatchability, blood components, thyroid hormone T4, and immunity to hatching chicks and reduce bacterial contamination of the eggshell surface of quail eggs.

Keywords: *Quail,Moringa oil, embryonic development , blood constituents , hatchability.*

INTRODUCTION

Microbial contamination of hatching eggs is a major concern for poultry producers as it causes poor hatchability and performance of chicks. It is clear that high standards of hygiene must be applied in hatcheries to reduce egg contamination, but additional disinfection of eggs is also necessary to reduce bacterial numbers (Cadirci *et al.,* 2009). The hatching eggs are disinfected to kill the microorganisms present on the surface of the shell. This allows the production of healthy poultry (Futura *et al.,* 1977). The main source of contamination of hatchery eggs is shell contact with soiled surfaces (Williams *et al.,* 1968; Mayes *et al.,* 1983). After laying the eggs most contamination of the shell occurs immediately; thus, the location and sanitation of the oviposition place is critical to cleaning the eggshell (Williams *et al.,* 1968; Smeltzer *et al.,* 1979). Surface bacteria can be transmitted to the interior of the egg through the

eggshell pores, which contain 7,000 to 17,000 pores (Williams *et al.,* 1968; Barbour *et al.,* 1984). It is common that microorganisms present on the shell, Escherichia coli, Salmonella enteritidis, Enterobacter, as well as yeasts and fungi (Al-Shammari *et al.,* 2015), may be a factor that significantly reduces hatching ability and may also cause mortality in chicks in the first few days after hatching (Koval *et al.,* 2003). An effective treatment that prevents the development of microorganisms on the shell is egg disinfection. The most common methods are fogging with formalin vapor, ultraviolet irradiation, and spraying with an antiseptic (Durmus *et al.,* 2012). A good disinfectant should be relatively inexpensive and odorless, effective against microorganisms, resistant to adverse environmental conditions, non-toxic and easily biodegradable to living organisms (Olesiak *et al.,* 2012). Formaldehyde, commonly used to disinfect eggs, is an irritant, highly toxic, and carcinogenic; it is also slowly biodegradable and therefore harmful to the natural environment (Rhomberg *et al.,* 2015; Whistler *et al.,* 1989). Although this method is effective in keeping incubation with low levels of contamination, it is important to highlight that formaldehyde is toxic, not only to birds but also to humans, and for this reason, it is increasingly being replaced in poultry production by disinfectants. It is generally believed that natural bioactive herbal compounds (from plants) are more acceptable and less dangerous than synthetic compounds and represent a rich source of potential disease-fighting agents. As a result, interest is increased in developing alternative methods for controlling microbial contamination; either by reducing or eliminating all rather than relying on synthetic pesticides. This method involves the use of products derived from plants, such as plant essential oils such as garlic and propolis oils, which have bactericidal effects (Aygun *et al.,* 2012 and Fouad *et al.,* 2018).

Moringa oleifera is the most widely cultivated species of the monophyletic family, Moringa that is native to the sub-Himalayan regions of India, Bangladesh, Pakistan and Afghanistan. For its multipurpose uses, it is often referred to as the miracle tree. Most of its parts are useful for a large number of applications (Posmontier *et al.,* 2011 and Vongsak *et al.,* 2013). 38 to 40% of the oil can be obtained from Moringa seeds (called ben oil from the high concentration of behenic acid present in the oil), the refined oil is as pure as any other vegetable oil (FAO, 1999). It has a flavor often described as mild and nutty and has a pale yellow colour. And its strong antioxidants that act as natural preservatives maybe the reason for its resistance to rancidity (Adetunji *et al.,* 2013). Its seed extracts have also been reported to exert in vitro bactericidal activity against Gram-negative and Gram-positive bacteria in raw water. Previous reports also revealed that Moringa seed extract contains a "flo" polypeptide that acts as a coagulant as well as an antibacterial effect on harmful bacterial strains (Suarez *et al.,* 2003). Several vitamins (A, E, folic acid, B2, B5, B6,) and minerals (Ca, Fe) have been indicated in Moringa (Bie *et al.,* 2017). It also has strong fungicidal and antimicrobial activity. It also has an inhibitory effect on the concentration of cholesterol in the blood (Ghasi *et al.,* 2000 and Yang *et al.,* 2006). It indicated that M. oleifera improves immunity, reduces Escherichia coli, and enhances Lactobacilli in chicken digestive system. M. oleifera improves the rate of food conversion and increases the immune reception of birds (Ghasi *et al.,* 2000).Moringa seed oil has a high content (80.4%) of polyunsaturated fatty acids (Anwar and Rashid, 2007 and Ogbunugafor *et al.,* 2011), and some researchers have discussed the antimicrobial and antioxidant effects of Moringa oleifera. Jabeen *et al.* (2008) reported that the antimicrobial properties of Moringa oleifera seed extracts may be due to the lipophilic compounds. These compounds may bind to the cytoplasmic membrane. The authors also suggested that Moringa oleifera seed extracts may contain antibiotic metabolites, such as carboxylic acid, 2,4-diacetyl phloroglucinol, and cell wall-degrading chitinases and enzymes.

This study evaluated the impact of spraying quail eggs with a solution of Moringa seed oil as a natural disinfectant to control microbial activity, physiological changes in embryonic development, hatching, blood components, hormones, immunity, and bacterial load on the surface of quail egg shells and the growth and development of chicks up to 14 days of age.

MATERIALS AND METHODS

This experiment was carried out at Poultry Experimental Station, Faculty of Agriculture, New Valley University, Egypt.

Preparation of solutions:

Moringa oil was obtained from the pressing process of Moringa seeds at the National Research Center in Egypt. A 2 ml Moringa oil solution /l was prepared by mixing 998 mL of 70% ethyl alcohol and 2ml of Moringa oil; a 4ml/l Moringa oil solution was prepared by mixing 996 mL of 70% ethyl alcohol and 4ml of Moringa oil; 6ml/l of Moringa oil solution was prepared by mixing 994 mL of 70% ethyl alcohol and 6ml of Moringa oil.

Experimental eggs:

Seven hundred and fifty fertilized eggs were obtained from a flock of Japanese quail aged 14 weeks and grew up on a commercial farm, in New Valley, Egypt. Eggs of Japanese quail were randomly and equally divided into five groups of 150 eggs each. Eggs from the first group were served as a control group (non–treated eggs). The second group was sprayed with the organic solvent 70% ethyl alcohol. The third group was sprayed with 2ml/l Moringa oil solution. The fourth group was sprayed with 4ml/l Moringa oil solution. The fifth group was sprayed with 6ml/l Moringa oil solution. The solutions were sprayed on the egg surfaces, using a hand sprayer, to cover the whole surface. The eggs were allowed to dry at 22°C for 10 minutes. 30 eggs from each group were labeled, then weighed at the beginning and on days 10 and 14 of incubation to estimate the egg weight loss ratio. Infertile eggs and eggs containing dead embryos were excluded from the calculation percentage of egg weight loss.

Incubation management:

Eggs were incubated in a commercial incubator at a temperature of 37.5°C and 65 relative humidity until day 14th of incubation then, were changed to 75% RH and 37 °C at the last three days. Eggs were automatically turned every two hours at 90 degrees.

Bacteriological examination:

Four eggs from each group were taken for bacteriological measurements. After 24 hours, 7th and 14th days of incubation. Each egg was placed immediately in a sterile bag containing 10 ml of sterile phosphate-buffered saline (PBS) with 7.2 pH. The egg-washing technique was carried out to recover the shell-associated bacteria for estimating the total viable bacterial count (TBC) and coliforms. Serial dilutions were made in PBS and then cultivated into sterile Petri plates (Gentry and Quarles, 1972 and Jones *et al.,* 2002). The plates were incubated at 37°C for 24 hours and at the end of incubation, the plates were removed and colonies were counted and multiplied by the dilution factor. Colonies were measured as cfu/egg (Özelik, 1992). Coliforms were determined with the most probable number technique. The incubated tubes that showed a yellow tint (acid production) and gas were considered to be positive.

Bacteriological count:

Aerobic plate counts (APC), total anaerobic counts, and total coliform counts were carried out according to American Public Health Association (A.P.H.A, 1985).

Estimation protocol:

At 14 days of incubation, 6 eggs from each group were taken for embryonic development examination. The percentages of embryo weight and albumen weight were estimated in relation to the egg weight. Embryo length, embryo shank length, shell thickness (mm) at 14 days, and egg weight loss at the 14th day of incubation were measured using a metal caliper digital and sensitive balance. The body weight of hatched chick, chick body length, and shank length were measured. After hatch, twelve chicks randomly selected- per each group were slaughtered for taking blood samples. The internal organs were weighed (residual yolk sac, intestine, liver, gizzard, and heart) and expressed as a percentage of the live body of the hatched chick. A portion of the fresh blood was used to determine red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), and white blood cells (WBCs). Serum was obtained from the blood samples by centrifugation for 15 minutes at 3000 rpm and stored at – 20 Cº until used in further analysis of blood constituents; blood biochemical parameters (total protein, albumin, and globulin (mg/dl)) in blood serum were determined by using the commercial kits (Biolabosa As. Frances). Blood hormones (thyroxine hormone (T4) and growth hormone (GH)) and immunity parameters; Immunoglobulin G (IgG) were determined by enzyme immunoassay using commercial kits (Monobind As. USA America).

Hatching parameters:

During 413 and 424 hours of incubation, eggs were checked individually every 6 hours for recording hatched chicks. After 17.7 days of incubation, all hatched chicks were removed from each hatch basket and weighed. Hatch time was monitored after the hatch of the first chick. Un-hatched eggs were opened to determine embryonic mortality. The hatchability of fertile eggs was calculated.

Chick performance procedure:

At the end of the incubation phase, 45 chicks per each group (15 chicks/pen) were kept till 14 days of age. Chicks were weighed and identified with a wing ring number. Chicks were raised (3 pens/group). A starter diet (24 % CP and 2,900 kcal of ME/kg) was provided ad libitum. 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). Moringa seeds oil contains all the fatty acids as in Moringa oleifera oil according to (Rahim, 2014) as shown in the following Table (1b).

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

Ingredients	**%**
Ground yellow corn	57.83
Soya bean meal (44%)	32.94
Fish meal (60.05%)	3.50
Corn gluten (62)	3.48
Dicalcium phosphate	0.33
Limetone	1.16
DL-Methionine	0.09
Lysine	0.07
Iodized sodium chloride	0.30
Minerals and vitamins premix	0.30
Calculated composition	
Crude protein (%)	24.00
ME (kcal/kg)	2900.00
Calorie/protein ratio (C/P	120.83
Calcium (%)	0.80
Phosphorus (%)	0.30

* *Each 3 kg of vit-mineral mixture contain: Vit A 10 m IU, Vit D3 1 m IU, Vit E 10 g, Vit k3 1 g, Vit B1 1g, Vit B2 4.0g, Vit B6 1.5g, Nicotinic acid 20g, Pantothenic acid 10g,vit B12 0.01g, Biotin 0.05g, Folic acid 30g, Choline chloride 50g, Iron 30g,Manganese 40g, Copper 3g, Iodine 0.45g, Zinc 45g and Selenium 0.1g.*

***ME was calculated according NRC (1994).*

Table (1b): Fatty acids composition of moringa seeds oils:

Type of fatty acids	**Carbon atoms**	**Moringa oleifera oil**
Saturated fatty acids (%):		
Myrstic	14:0	-
Palmatic	16:0	6.58
Stearic	18:0	5.33
Arochidic	20:0	3.44
Behenic	22:0	6.69
Lignoceric	24:0	1.07
Total saturated fatty acids (TSFA)		23.11
Unsaturated fatty acids (%):		
Palmitoleic	16:1	1.66
Oleic	18:1	72.42
Linoleic	18:2	0.65
Linolenic	18:3	0.17
Eicosaenoic	20:1	1.99
Erucic	22:1	-
Total unsaturated fatty acids (TUSFAs), %		76.89
Monounsaturated fatty acids (MUSFAs), %		76.07
Polyunsaturated fatty acids (PUSFAs), %		0.82
Total fatty acids (TFAs), %		100

All chicks were daily exposed to 23 hours lighting with darkness for one and the temperature was set at 33°C. At 7 days and 14 days, all chicks were individual weight. For each chick, live body weights were recorded.to calculate body weight gain (BWG). Feed intake (FI) was recorded for each replicate and herewith feed conversion ratio (FCR) as g feed/g BWG was calculated. At the end of 14 days, six - randomly selected- chicks per each group were slaughtered for obtaining blood samples. A portion of the fresh blood was used to determine red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV),

and white blood cells (WBCs). Serum was obtained from the blood samples by centrifugation for 15 minutes at 3000 rpm and stored at – 20 C° until used in further analysis of blood constituents; blood biochemical parameters (total protein, albumin, and glucose Globulin (mg/dl)) in blood serum were determined by using the commercial kits (Biolabosa As. Frances). Blood hormones (thyroid hormone (T4) and growth hormone (GH)) and immunity parameter; Immunoglobulin G (IgG) were determined by enzyme immunoassay using commercial kits (Monobind As. USA America). Intestinal aerobic and anaerobic microflora counts were determined. Total anaerobic count, total coliform count, and aerobic plate count (APC) were carried out according to American Public Health Association (A.P.H.A, 1985).

Statistical analysis:

Data obtained were analyzed using the GLM procedure of Statistical Analysis System (SAS, 2002), using one-way ANOVA as in the following model: Yik= µ+ Ti + eik Where Y is the dependent variable; µ is the general mean; T is the effect of treatments; and e is the experimental random error. The differences among experimental groups were separated by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

There was a significant increase in the percentages of embryo weight and embryonic length on the 14th day of the incubation period, in addition to the increase in the chick body weight (g), chick length (cm), and chick shank length at hatch ($P<0.05$) for eggs treated with Moringa oil compared with control eggs (Table 2,3). Eggs sprayed with a solution of Moringa oil (6 ml/liter) had the highest values on the 14th day of incubation and at hatch. There was a significant ($P<0.05$) increase in the consumption of albumen. Consequently, an increase in embryo weight was observed on the fourteenth day of the groups treated with a solution of Moringa oil than those of the control group. On the other hand, there was a significant ($p <0.05$) decrease in albumen on the 14th day of incubation (Table 2). The lowest albumen content was found in the eggs treated with 6 ml Moringa oil solution /liter of, followed by those treated with 4 and 2 ml/liter, respectively as compared with the control group.

Egg weight loss on 14th day of the incubation phase is presented in (Table 2). Results showed that egg loss percentages significantly ($P<0.05$) differed between 9.51 and 10.82 % among all groups on the 14th day. The egg weight losses of all Moringa oil solution treatment groups were significantly ($P<0.05$) lower than those of the untreated groups. Eggshell thickness was a significant decrease ($P<0.05$) on the 14th day of incubation in eggs sprayed with Moringa oil solution (Table 2).

The effect of Moringa oil solution on the length of the incubation period (hatch time/hrs) was shown in (Table 3). The results showed that the treated eggs had shorter incubation times than those of the untreated eggs. Chicken eggs were sprayed with a solution of Moringa oil (2, 4, 6 ml / L) and recorded shorter incubation times (419, 415, and 413 hrs), respectively as compared with that of the 70% ethyl alcohol spray (423 hrs) and control group (424 hrs). Moringa seeds contain between 330 and 410g/kg of oil. Moringa seed oil is highly resistant to oxidation, which could explain why several industrial uses such as cosmetics. It is also used in cooking oil, and in perfumery due to its high odor retention capacity (Makkar and Becker, 1999 and Francis *et al.,* 2005). Moringa oleifera is a good source of vitamins (A and E), amino acids, and low levels of anti-nutritional compounds (Olugbemi et al; 2010a and Yang *et al.,* 2006). Moringa oleifera boosts the immune system (Jayavardhanan *et al.,* 1994; Fuglier, 1999 and Olugbemi *et al.,* 2010).

The seeds of Moringa oleifera contain 19 to 47 percent of the oil (Ahmad *et al.,* 1989). Commercially known as"ben oil", it is similar to olive oil and is rich in Palmitic, Stearic, Behenic and Oleic Acids (Nautiyal *et al.,* 1987). The oil is clear, odorless, and resists rancidity (Ferro *et al.,* 1970). This oil contains about 75% oleic acid, which is a monounsaturated fatty acid that is less vulnerable to oxidative stress than unsaturated fats. Oleic acid has the ability to reduce inflammation in the system, as oleic acid appears to be a major preventive factor in reducing levels of cardiovascular disease, and breast and skin cancer (Pauwels, 2011). The oil has high antioxidant properties, making it a valuable source of Vitamin A, C, and E, and one of the highest sources of natural antioxidants (Dogra *et al.,* 1975). These oils possess antioxidant, anti-bacterial, anti-fungal, and antipyretic properties (Rajib Singha, 2010).

Moringa seeds are also rich in methionine, cysteine, carotenoids, and calcium and are used as a vitamin A supplement. Also, they have been reported to have antioxidant, hepatoprotective, hypoglycemic, and antihypertensive effects. It has the potential to be an alternative fodder for animals during periods of drought, and apart from the plant it is a good source of vitamins and amino acids, and has 8 medicinal uses (Makkar and Bekker 1999 and Francis et al, 2005).

Moringa oleifera has also been used in the treatment of several diseases (Pal *et al.,* 1995, Makonnen *et al.,* 1997, Gbasi et al, 2001 and Matthew *et al.,* 2001) including obesity and heart disease due to hypocholesterolemia (Gbasi *et al.,* 2001 and Olugbemi *et al.,* 2010). Moringa species are rich in compounds containing the simple sugar, rhamnose, and are rich in a unique group of compounds called isothiocyanates and glucosinolates (Bennett *et al.,* 2003 and Fahey *et al.,* 2001). These results may be due to the good health status of embryos which may be caused by treatment with Moringa oil solution as a natural disinfectant as reported by Fouad *et al.,* (2018), who found that spraying Japanese quail eggs with natural oils as natural disinfectants (pre-incubation) is a good way to improve embryonic development.

Egg weight loss is an important incubation factor. Loss of moisture was undesirable for normal embryonic development (Geng and Wang 1990). The egg weight loss percentage as a result of treating eggs with an antiseptic is reasonable because the disinfectants may affect the cuticle layers and the porosity of the shell. This view was confirmed by Brake and Sheldon (1990), who noted that any alteration or removal of the cuticle by disinfectants may have a significant effect on egg weight loss and hatchability. This result can be explained by the light of the clogging of egg pores due to the oily nature of these disinfectants, which reduced the evaporation of water vapor and the percentage of egg loss in weight (Shaheen and Iman 2014). The findings of Fouad *et al.,* (2018), showed that the egg weight losses of the treated groups by natural oils solution were significantly lower (P> 0.05) when compared with those of untreated groups. This can be explained by a reduction in water loss through the coating of egg pores after spraying with natural oils solution. Also, they noted that the eggshell thickness (mm) of eggs treated with natural oils as natural disinfectants was significantly reduced on the 14th day of incubation compared to untreated. This may be due to the interaction between the natural oils and the eggshell that changes its properties, which may have some physical changes in the morphology or cause the eggshell to thin.

Hatchability of fertile eggs:

The percentage of hatchability had significantly increased for all treated groups by spraying with Moringa oil solution as a natural disinfectant compared to untreated groups (Table 3). The highest percentage was observed in eggs sprayed with 6 ml / L Moringa oil solution compared with the lowest one in control. Spraying fertile eggs with either 4 or 6 ml / L Moringa oil solution resulted in increased hatchability by about 16.43 and 11.1%, respectively than those of the control. Consequently, embryonic mortality rate was significantly differed between groups treated with Moringa oil solution and untreated eggs (Table 3), the lowest one was observed in eggs sprayed by 4 and 6 ml / L Moringa oil solution than those of the untreated groups. These results are consistent with the results reported by Mona, 2011, Fouad and Abdel-Hafez (2017) and Fouad *et al.* (2018), who reported that the shortest incubation period was observed for chicks produced from eggs treated with natural disinfectants. This is in harmony with those of Dehghani *et al.* (2018) who stated that these plant essential oils can replace antibiotic growth factors without having any negative effect on quail health. The findings of Yildirim *et al.* (2003) showed that essential oil could be considered as a potential alternative to a disinfectant for hatching eggs without adversely affecting the hatching of quail eggs and there was a significant effect on hatchability with significantly higher Mortality after formaldehyde treatment compared to essential oil treatment. Fouad *et al.* (2018), who reported that spraying Japanese quail hatching eggs with natural oils as natural disinfectants (before incubation) is a good method for improving hatchability and reducing embryo mortality.

Blood constituents:

the results in Tables 4, 5 showed a significant (P<0.05) increase in the hematological parameters, total protein, albumin, globulin, hormones (T4 and GR. H), and IgG of chicks hatched from eggs sprayed by L Moringa oil solution as compared to the control and Ethyl alcohol 70% sprayed groups.

Spraying fertile eggs with either 4 or 6 ml / L Moringa oil solution led to an increase in RBCs by 14.7 and 21.1 %, Hb by 22.5 and 31.5 %, PCV% by 12.2 and 15.6 %, total protein by 21.4 and 28.6%, albumin by 39.5 and 54.5 %, globulin by 13.9 and 17.1 %, T4 by 8.9 and 14.5 %, growth hormone by 21.1 and 49.2 % and IgG by 0.44 and 0.84 of the control value, respectively. There was a non-significant difference in counts of different white blood cells (%) in hatched chicks (Table 4). Fouad *et al.* (2018), showed that spraying Japanese quail eggs with natural oils as a natural disinfectant (pre-incubation) is a good way to improve blood components (hematological parameters, total plasma protein, albumin, T4 hormones, and immunity G (IgG)) hatching chicks. Sayed Ahmed *et al.,* 2019, showed that total blood protein (TP) and globulin (Glb), were significantly increased (P≤ 0.05) due to dietary Moringa oil compared to the control group. However, the Glb level has been used as an indicator of increased

immunity and a source of antibody production. Increased Glb concentration may be an indicator of increased immunity in the liver and it will be able to synthesize a sufficient amount of Glb for immunologic action as reported by Sunmonu and Oloyede (2007).

Internal organs weight:

Relative weights of Yolk residual, intestine, liver, gizzard, and heart of Japanese quail chicks are presented in (Table 6). Intestine, liver, gizzard and heart relative weight for chicks hatched from eggs sprayed with 4 and 6 ml/ L Moringa oil solution were higher than those of eggs sprayed with Ethyl alcohol 70% or the control group. Furthermore; the relative weight of residual yolk for chicks hatched from eggs sprayed with 4 and 6 ml / L Moringa oil solution was lower than the control group. These results are in agreement with the finding of (Fouad and Abdel-Hafez, (2017); Fouad *et al.,* 2018) who showed that spraying Japanese quail eggs with natural disinfectants or natural oils as natural disinfectants (pre-incubation) is a good way to improve Internal organs of chicks at hatch. Sayed Ahmed *et al.* (2019), showed that it can be assumed that dietary Moringa oil supplementation may help overcome the negative effects of heat stress on growth performance and carcass yield.

Growth performance:

The averages of body weight, body weight gain, feed intake, and feed conversion of chicks of Japanese quail chicks are presented in (Table 7). Statistical analysis showed that average body weight, feed intake and body weight gain for chicks hatched from eggs treated with Moringa oil solution were significantly higher ($P<0.05$) than those for eggs sprayed with Ethyl alcohol 70% or the control group. Also, results showed an improvement in feed conversion rate. Spraying fertile eggs with either 4 or 6 ml / L Moringa oil solution led to an increase in body weight by 18.2 and 21.9 %, feed intake by 11.4 and 14.8 %, body weight gain by 21.4 and 25.8%, of the control group, respectively. Moringa seeds have been suggested as a viable alternative source of proteins, vitamins and minerals for feeding poultry, Church, (1991). Nworgu and Fasogbon (2007) observed an increase in body weight in the growth of chickens fed diets containing moringa seed meal. On the other hand, Du et al. (2007) no significant difference was observed in the growth performance of 3-week-old broiler chickens (Arbor Acres) fed diets supplemented with M. oleifera seed meal.

Moringa seeds are a good source of fats, protein, antioxidants and minerals (Mg and Zn), so they can overcome malnutrition due to a deficiency of micronutrients, Compaore *et al.,* (2011). Moringa seed oil contains a high content (80.4%) of polyunsaturated fatty acids (Anwar and Rashid, 2007 and Ogbunugafor *et al.,* 2011). The results of Sarah, (2014) showed that moringa seed meal supplementation remarkably improved growth rate of unsexed Ross 308 broiler chickens aged one to 21 days. Spraying Japanese quail eggs with natural disinfectants or natural oils as natural disinfectants (pre-incubation) is a good way to improve the growth performance (feed intake, body weight and body weight gain) of chicks at 14 days of age and improved feed conversion as reported by (Fouad and Abdel-Hafez, 2017 and Fouad *et al.,* 2018). Sayed Ahmed *et al.,* (2019) found that the dietary Moringa oil supplementation may help overcome the negative effects of heat stress on growth performance

Blood constituents:

Results in Tables 8,9 showed a significant ($P<0.05$) increase in the hematological parameters, total protein, albumin, globulin, hormones (T4 and GH), and IgG of chicks at 14 days of age hatched from eggs sprayed by Moringa oil solution as compared to the control and Ethyl alcohol 70% sprayed groups. There was a non-significant difference in counts of different white blood cells (%) in chicks (Table 8). Sayed Ahmed *et al.,* (2019) noted that total blood protein and globulin were significantly increased ($P\leq0.05$) due to dietary Moringa oil compared to the control group. However, the globulin level has been used as an indicator of increased immunity and a source of antibody production. Increased globulin concentration may be an indicator of increased immunity in the liver and it will be able to synthesize a sufficient amount of globulin for immunologic action as reported by Sunmonu and Oloyede (2007).

Microbiological study:

Natural disinfectants of Moringa oil solution had a significant influence on total bacterial count TBC and Total coliform count compared to the control group after 24 hours, 7th and14th days of incubation (Table 10.11). The best significant results of TBC and Total coliform Count after 24 hours, 7 th and14 th days of incubation were observed for eggs sprayed with 4 and 6 ml Moringa oil solution / L. The intestinal microbial counts of chicks at 14 days of the age of Japanese quails are presented in (Table 12). The intestinal total anaerobic, total coliform, and aerobic counts decreased for chicks of treated eggs in comparison with the control group. Madsen *et al.,* (1987) reported that the use of Moringa oleifera seeds reduced the bacterial count of turbid Nile water in Sudan by 1-4 logarithmic units (90-99.9%) during the

first to two hours of treatment. Moreover, Walter *et al.* (2011) reported that Moringa oleifera and Moringa stenuptala methanol and N-hexane seed extracts produced an inhibitory effect on Salmonella typhi, Vibrio cholera and Escherichia coli, which commonly cause waterborne diseases.

Moringa seeds also possess antimicrobial properties (Olsen, 1987 and Madsen *et al.,* 1987). Also, Broin (2007) showed that the recombinant protein in seeds is able to flocculate cells of both Gram-positive and Gram-negative bacteria. In this case, the microorganism can be removed by settling in the same way as colloid removal in properly coagulated and flocculated water (Casey, 1997). On the other hand, the seeds may also act directly on the microorganism and lead to growth inhibition. Antimicrobial peptides disrupt the cell membrane or inhibit essential enzymes (Silvestro *et al.,* 2000 and Suarez *et al.,* 2003). The findings of Sutherland *et al.,* (1990) showed that Moringa seeds could inhibit the replication of bacteriophages. The seeds' antimicrobial effects are attributed to the 4 (α-L-rhamnosyloxy) benzyl isothiocyanate complex (Eilert *et al.,* 1981).

Also, some of the compounds isolated from moringa preparations that have been reported to have antihypertensive, anticancer and antibacterial activity include 4-(L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate (Daxenbichler *et al.,* 1991; Fahey *et al.,* 2001; Bennett *et al.,* 2003; Mekonnen and Dräger, 2003). The antioxidants of these 10 compounds (Win and Jongen, 1996). Moringa seeds contain glucosinolate that are produced by hydrolysis 4- (a-L-rhamnosyloxy) -benzyl isothiocyanate, an active bactericide and fungicide (Grubben and Denton, 2004).Moringa oleifera seeds are good and safe for treating water compared to synthetic (alum) chemical compounds that may be carcinogenic (Ayotond *et al.,* 2011). Fouad *et al.,* 2018, reported that spraying Japanese quail eggs with natural oils as natural disinfectants (before incubation) is a good way to reduce bacterial contamination of the surface of quail eggs' shells.

CONCLUSION

Finally according to the results of this study it is clear that spraying Japanese quail eggs with a solution of moringa oil as a natural disinfectant (pre-incubation) is a good way to improve embryonic development, hatchability, blood components, thyroid hormone T4 and immunity to hatching chicks and reduce bacterial contamination of the eggshell surface of quail eggs.

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Table (2): Effect of spraying Japanese quail hatched eggs by moringa oil on embryo parameters at 14th day of incubation.

Traits / Treatments	Initial egg weight (g)	Embryo weight %	Albumen weight %	Shank length(mm)	Body length (mm)	Egg shell thickness (mm)	Egg weight loss(%)
Control (Non–treated eggs)	12.58	36.78^{d}	1.34^{a}	10.05^{c}	66.44^{d}	19.00^{a}	10.82^{a}
Spraying by ethyl alcohol 70 %	12.63	37.21^{d}	1.24^{a}	10.15^{c}	66.98^{d}	19.22^{a}	10.77^{a}
Spraying by moringa oil (2 ml/L)	12.67	41.36^{c}	0.33^{b}	14.30^{b}	76.34^{c}	17.44^{b}	9.83^{b}
Spraying by moringa oil (4 ml/L))	12.60	42.53^{b}	0.19^{c}	14.77^{ab}	79.18^{b}	17.00^{bc}	9.73^{c}
Spraying by moringa oil (6 ml/L)	12.59	45.37^{a}	0.10^{c}	15.24^{a}	81.36^{a}	16.66^{c}	9.51^{c}
Pooled SEM	0.19	1.41	0.12	0.62	1.76	0.82	0.14
P value	0.631	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

$^{a,b,c,\ d}$ *Means with the different letters in the same column are significantly different (P<0.05)*

Table (3): Effect of spraying Japanese quails hatched eggs by moringa oil on hatched chick parameters.

Traits / Treatment groups	Chick body weight (gm)	Chick body length (mm) (1d)	Chick shank length (mm) (1d)	Hatchability of fertile eggs (%)	Embryonic mortality of fertile eggs (%)	Hatch time (hrs)
Control (Non–treated eggs)	9.26^{c}	107.00^{d}	19.00^{d}	76.64^{d}	23.35^{a}	424.00^{a}
Spraying by ethyl alcohol 70%	9.12^{c}	106.33^{d}	18.96^{d}	76.20^{d}	23.79^{a}	423.00^{a}
Spraying by moringa oil (2 ml/L)	9.69^{b}	112.00^{c}	20.70^{c}	82.52^{c}	17.47^{b}	419.00^{b}
Spraying by moringa oil (4 ml/L))	9.93^{ab}	116.00^{a}	21.03^{b}	85.14^{b}	14.85^{c}	415.00^{c}
Spraying by moringa oil (6 ml/L)	10.00^{a}	118.33^{a}	21.60^{a}	89.23^{a}	10.76^{d}	413.00^{c}
Pooled SEM	0.29	2.52	0.14	1.95	1.95	2.42
P value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

$^{a,b,c,\ d}$ *Means with the different letters in the same column are significantly different (P<0.05)*

Table (4): Effect of spraying Japanese quails hatched eggs by moringa oil on hematological traits of hatched chicks.

Traits / Treatments	RBC (10^6/mm^3)	HB (g/dl)	PCV %	WBC (10^3/mm^3)	Lymphocytes (%)	Heterophils (%)	Monocytes (%)	Eosinophils (%)
Control (Non–treated eggs)	1.90^{d}	9.76^{c}	22.20^{d}	47.58	53.04	42.0	3.09	1.86
Spraying by ethyl alcohol 70%	1.87^{d}	9.40^{c}	21.86^{d}	47.64	53.04	41.99	3.05	1.91
Spraying by moringa oil (2 ml/L)	2.04^{c}	11.43^{b}	23.20^{c}	47.90	53.01	42.03	3.08	1.87
Spraying by moringa oil (4 ml/L)	2.18^{b}	11.96^{b}	24.90^{b}	47.90	52.95	42.04	3.02	1.98
Spraying by moringa oil (6 ml/L)	2.30^{a}	12.83^{a}	25.66^{a}	47.92	52.95	42.07	3.03	1.94
Pooled SEM	0.05	0.51	0.51	1.43	0.19	0.26	0.30	0.35
P value	0.0001	0.0001	0.0001	0.8011	0.6035	0.8803	0.9336	0.9008

$^{a,b,c,\ d}$ *Means with the different letters in the same column are significantly different (P<0.05).*

Table (5): Effect of spraying Japanese quails hatched eggs by Moringa oil on biochemical constituents, hormones and immunity parameters of hatched chick.

Traits / Treatments	biochemical constituents			hormones		immunity parameter
	Total protein (g/dl)	Albumin (g/dl)	Globulin(g/dl)	T4 (ng/ml)	GR .H (ng/ml)	IgG (mg/100 ml)
Control(Non–treated eggs)	2.80^{d}	0.86^{b}	1.94^{b}	8.96^{d}	0.71^{d}	908.00^{cd}
Spraying by Ethyl alcohol 70%	2.70^{d}	0.80^{b}	1.90^{b}	8.86^{d}	0.70^{d}	907.33^{d}
Spraying by Moringa oil (2 ML/L)	3.13^{c}	0.96^{b}	2.17^{a}	9.20^{c}	0.74^{c}	910.00^{bc}
Spraying by Moringa oil (4 ML/L))	3.40^{b}	1.20^{a}	2.20^{a}	9.76^{b}	0.86^{b}	912.00^{b}
Spraying by Moringa oil (6 ML/L)	3.60^{a}	1.33^{a}	2.27^{a}	10.26^{a}	1.06^{a}	915.66^{a}
Pooled SEM	0.21	0.22	0.24	0.37	0.03	2.79
P value	0.0001	0.0005	0.0068	0.0001	0.0001	0.0001

a,b,c, d *Means with the different letters in the same column are significantly different (P<0.05).*

Table (6): Effect of spraying Japanese quails hatched eggs by moringa oil on some relative internal organs to carcass of hatched chicks.

Traits / Treatments	Yolk residual %	Intestinal %	Liver %	Gizzard %	Heart %
Control (Non–treated eggs)	9.27	2.66^{cd}	2.39^{c}	4.40^{c}	0.65^{c}
Spraying by ethyl alcohol 70%	9.35	2.64^{d}	2.38^{c}	4.36^{c}	0.64^{c}
Spraying by moringa oil (2 ml/L)	8.94	2.74^{bc}	2.46^{bc}	4.46^{b}	0.78^{b}
Spraying by moringa oil (4 ml/L)	8.74	2.76^{ab}	2.52^{ab}	4.58^{ab}	0.83^{ab}
Spraying by moringa oil (6 ml/L)	8.05	2.84^{a}	2.56^{a}	4.73^{a}	0.87^{a}
Pooled SEM	1.09	0.08	0.07	0.17	0.08
P value	0.08	0.0042	0.003	0.001	0.0001

a,b,c, d *Means with the different letters in the same column are significantly different (P<0.05).*

Table (7): Effect of spraying Japanese quails hatched eggs by moringa oil on some performance parameters of chicks at 14 days of age.

Traits / Treatments	Initial chick weight (g)	Final body weight at 14 d (g)	Feed intake (g)	Body weight gain (g)	Feed conversion (g feed/g gain)
Control (Non–treated eggs)	9.53	62.83^{d}	78.66^{c}	53.30^{d}	1.47^{a}
Spraying by Ethyl alcohol 70%	9.53	62.24^{d}	77.66^{c}	52.70^{d}	1.47^{a}
Spraying by Moringa oil (2 ML/L)	9.54	72.93^{c}	86.66^{b}	63.38^{c}	1.36^{b}
Spraying by Moringa oil (4 ML/L))	9.53	74.26^{b}	87.66^{b}	64.73^{b}	1.35^{b}
Spraying by Moringa oil (6 ML/L)	9.53	76.62^{a}	90.33^{a}	67.09^{a}	1.34^{b}
Pooled SEM	0.29	1.16	2.28	1.34	0.02
P value	09999 (NS)	0.0001	0.0001	0.0001	0.0001

a,b,c, d *Means with the different letters in the same column are significantly different (P<0.05).*

Table (8): Effect of spraying Japanese quails hatched eggs by moringa oil on hematological traits of chicks at 14 days of age.

Traits / Treatments	RBC(10^6/ mm^3)	HB(g/ dl)	PCV %	WBC(10^3/ mm^3)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)
Control (Non–treated eggs)	2.36^{d}	15.76^{c}	37.06^{d}	48.58	52.33	42.96	3.19	1.50
Spraying by Ethyl alcohol 70%	2.343^{d}	15.46^{c}	36.13^{d}	48.90	52.00	43.05	3.48	1.45
Spraying by Moringa oil (2 ML/L)	2.54^{c}	16.50^{bc}	38.36^{c}	48.90	52.56	42.83	2.68	1.92
Spraying by Moringa oil (4 ML/L))	2.75^{b}	17.03^{ab}	39.80^{b}	49.31	52.61	42.84	2.72	1.81
Spraying by Moringa oil (6 ML/L)	2.84^{a}	17.93^{a}	42.03^{a}	49.59	52.56	42.80	2.96	1.66
Pooled SEM	0.05	1.33	1.84	1.01	1.35	0.9	1.12	0.78
P value	0.0001	0.0005	0.0001	0.5597	0.3513	0.9493	0.7107	0.2352

a,b,c, d *Means with the different letters in the same column are significantly different (P<0.05).*

Table (9): Effect of spraying Japanese quails hatched eggs by moringa oil on biochemical constituents, hormones and immunity parameters of chicks at 14 days of age.

Traits / Treatments	Biochemical constituents			Hormones		Immunity parameter
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	T4 (ng/ml)	GH (ng/ml)	IgG(mg/100 ml)
Control (Non–treated eggs)	3.13^{c}	1.26^{c}	1.86^{bc}	9.83^{c}	0.77^{cd}	916.3^{c}
Spraying by Ethyl alcohol 70%	3.06^{c}	1.23^{c}	1.83^{c}	9.80^{c}	0.760^{d}	916.00^{c}
Spraying by Moringa oil (2 ML/L)	3.43^{b}	1.433^{b}	2.00^{b}	10.33^{b}	0.79^{c}	920.00^{b}
Spraying by Moringa oil (4 ML/L))	3.66^{b}	1.46^{a}	2.20^{a}	10.66^{a}	0.88^{b}	922.00^{b}
Spraying by Moringa oil (6 ML/L)	3.93^{a}	1.63^{a}	2.30^{a}	10.80^{a}	1.10^{a}	925.6^{a}
Pooled SEM	0.23	0.17	0.19	0.27	0.029	2.79
P value	0.0001	0.0002	0.0001	0.0001	0.0001	0.0001

$^{a,b,c,\,d}$ *Means with the different letters in the same column are significantly different (P<0.05)*.

Table (10): Effect of spraying Japanese quails hatched eggs by moringa oil on total bacterial count (TBC) of egg shell surface (X 10^3 CFU /egg) at 1, 7, 14 days of incubation.

Traits / Treatments	T.B.C (X 10^3 CFU /egg)		
	1 day	7 day	14 day
Control (Non–treated eggs)	30.58^{a}	34.43^{a}	34.96^{a}
Spraying by Ethyl alcohol 70%	29.94^{a}	34.32^{a}	34.39^{a}
Spraying by Moringa oil (2 ML/L)	16.15^{b}	15.70^{b}	15.14^{b}
Spraying by Moringa oil (4 ML/L))	15.07^{b}	14.29^{c}	13.61^{c}
Spraying by Moringa oil (6 ML/L)	13.43^{c}	12.58^{d}	12.01^{d}
Pooled SEM	1.12	1.70	1.34
P value	0.0001	0.0001	0.0001

$^{a,b,c,\,d}$ *Means with the different letters in the same column are significantly different (P<0.05)*.

Table (11): Effect of spraying Japanese quails hatched eggs by moringa oil on total coliform count (TCC) of egg shell surface (X 10^3 CFU /egg) at 1, 7, 14 days of incubation .

Traits / Treatments	TCC (X 10^3 CFU /egg).		
	1 day	**7 day**	**14 day**
Control (Non–treated eggs)	4.10[a]	4.51[a]	6.76[a]
Spraying by Ethyl alcohol 70%	3.85[a]	4.30[a]	6.39[a]
Spraying by Moringa oil (2 ML/L)	2.96[b]	2.79[b]	2.5[b]
Spraying by Moringa oil (4 ML/L))	2.18[c]	1.89[c]	1.80c
Spraying by Moringa oil (6 ML/L)	2.03[c]	1.84[c]	1.77[c]
Pooled SEM	0.83	0.93	0.91
P value	0.0003	0.0001	0.0001

[a,b,c, d] *Means with the different letters in the same column are significantly different (P<0.05).*

Table (12): Effect of spraying Japanese quails hatched eggs by moringa oil on total anaerobic, total coliform and aerobic count of bacteria in chick intestine.

Traits / Treatments	Total anaerobic count X 10^3	Total coliform Count X 10^3	Aerobic plate count X 10^3
Control (Non–treated eggs)	0.31[a]	3.40[a]	4.41[a]
Spraying by Ethyl alcohol 70%	0.30[a]	3.31[a]	4.23[a]
Spraying by Moringa oil (2 ML/L)	0.09[b]	2.78[b]	3.03[b]
Spraying by Moringa oil (4 ML/L))	0.07[bc]	2.22[c]	1.30[c]
Spraying by Moringa oil (6 ML/L)	0.06[c]	1.16[d]	0.09[d]
Pooled SEM	0.02	0.54	0.77
P. Value	0.0001	0.0001	0.0001

[a,b,c, d] *Means with the different letters in the same column are significantly different (P<0.05).*

تأثير رش محلول زيت المورينجا على نمو الجنين، نسبه الفقس والعوامل الفسيولوجية ونمو الكتاكيت بعد الفقس والتلوث البكتيري لبيض السمان المخصب

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يحتوي زيت المورينجا على نشاط مضاد للجراثيم قوي ضد مجموعة واسعة من البكتيريا سالبة الجرام وموجبة الجرام بالإضافة إلى خصائصه المضادة للفطريات. هدفت هذه التجربة الى دراسة تاثير رش بيض تفريخ السمان الياباني بمحلول زيت المورينجا على التطور الجنيني، والصفات الفسيولوجية، ونمو الكتاكيت بعد الفقس، والحمل البكتيري على سطح قشر البيض. اشتملت هذه التجربة على عدد 750 بيضة سمان قسمت بالتساوي إلى خمس مجموعات تجريبية. المجموعة الأولى هي المجموعة الكنترول (بيض غير معالج) و المجموعة الثانية مجموعة كنترول تم رشها بمذيب عضوي ، 70٪ كحول إيثيلي. تم رش بيض المجموعات الثالثة والرابعة والخامسة بمحلول زيت المورينجا 2 و 4 و 6 مل / لتر على التوالي. اوضحت نتائج الدراسة وجود زيادة معنوية فى وزن وطول الجنين وطول عظمة الساق للجنين وايضا وزن الكتاكيت الفافسةوطول الجسم و عظمة الساق، كما زادت نسبة التفريخ من البيض المخصب فى البيض المعامل بمحلول زيت المورينجا مقارنة باللكنترول فى حين انخفض كل من وقت الفقس ونسبة الاجنة الميتة وزن الالبيومين كنسبةمئوية من وزن البيضة وقل سمك قشرة البيضة للبيض ونسبة الفقد المائى عند عمر 14 يوم من التفريخ للبيض المعامل برش محلول زيت الثوم بالمقارنة بالبيض الغير المعامل .تحسنت معنويا عدد كرات الدم الحمراء واليموجلوبين و pcv ،وبروتينات الدم الكليه والالبيومين والجلوبيولين وهرمون الغدة الدرقيةT4 وجلوبيولينات المناعه (IgG) بشكل ملحوظ ($P < 0.05$) في عمر 1 و 14 يومًا. ازداد عدد خلايا الدم البيضاء بشكل طفيف استجابة لمحلول زيت المورينجا. كما تحسنت مكونات الذبيحة للكتاكيت الفافسة وتحسنت صفات النمو للكتاكيت (وزن الجسم ووزن والعلف المستهلك ووزن الجسم المكتسب ومعدل التحويل الغذائى) عندعمر 14 يوم , في حين وجد انخفاض معنوى فى نسبة وزن الصفار المتبقى للكتاكيت الفافسه للبيض المعامل بالمقارنه بالكنترول . رش البيض بزيت الثوم كان له تأثير كبير حيث انخفض العدد البكتيرى الكلى معنوي TBC و coliforms على سطح قشر البيض بعد 24 ساعة و 7 و 14 يومًا من الحضانة مقارنة بمجموعات التحكم.كما انخفض العدد الكلي للبكتيريا الهوائية واللاهوائية في امعاء الكتاكيت والعدد الكلي للبكتيريا القولونية عند عمر 14 يوم. واخيرا يعتبر رش بيض السمان الياباني بمحلول زيت المورينجا كمطهر طبيعي (قبل وضع البيض فى المفرخه) طريقة جيدة لتحسين التطور الجنيني ، ونسبه الفقس وصفات الدم وهرمون الغدة الدرقية T4 ، والمناعه للكتاكيت الفاقسه وخفض التلوث البكتري على سطح قشره بيض التفريخ للسمان اليابانى.

الكلمات المفتاحية: *السمان ، زيت المورينجا ، التطور الجنيني ، مكونات الدم ، نسبة التفريخ*