



EFFECT OF THE USE OF IMMERSION and INJECTION METHODS FOR EGG HATCHING OF BROILER BREEDERS IN THE AQUATIC EXTRACTS OF SOME PLANT SEEDS AS EARLY FEEDING

K.J. Al-Asadi^{1*} and B.M. Ibrahim²

¹Department of Animals Services, Directorate of Agriculture in Dhi Qar Governorate, Iraq.

²Department of Animal Production, Engineering Sciences Agricultural, University of Baghdad, Iraq.

Abstract

This study was conducted in Al-Anwar hatchery - Babylon governorate for periods from 5/4/2019 to 27/4/2019 with the aim of showing the effect of applying the method of immersion and injection with aqueous extracts for some plant seeds (fenugreek, oats and basil) in some characteristics of hatching and hatched chicks. A total of 1050 eggs were used for hatching and were randomly divided into seven treatments with three replicates (50 eggs / replicate). Hatching eggs were immersed in aqueous extracts before incubation for 1 minute, while they were injected on the 17th day with amniotic fluid in a quantity of 0.2 ml. The experiment treatments were designed as follows: Treatment 1st, Control group (T_c) and treatments 2nd, (T_{DF}) (Dipping fenugreek) 3rd, (T_{IF}) (Injection fenugreek) Included immersion and injection of hatching eggs with the aquatic extract of the fenugreek seeds, treatments 4th, (T_{DO}) (Dipping oat (5th, T_{IO}) (Injection oat (Included immersion and injection of hatching eggs with the aquatic extract of the oat seeds, treatments 6th (T_{DB}) (Dipping basil) 7th (T_{IB}) (Injection basil) Included immersion and injection of hatching eggs with the aquatic extract of the basil seeds, the concentration of all extracts was 6% of treatments', respectively. The results of the current study indicated: The use of the method of immersion in the aqueous extract of fenugreek and basil seeds resulted in a significant improvement in chicks weight and hatching window, deterioration in most characteristics of hatching and hatched chicks for the treatment of basil extract injection (T_{IB}) compared to control. In conclusion, that the application of immersion method for hatching eggs of broiler breeders with the aqueous extract of fenugreek and basil seeds has improved most of the characteristics of hatching and hatched chicks.

Key words: Fenugreek, oats, basil, embryo, *in ovo*.

Introduction

In ovo Feeding is one of the recent trends that have emerged recently to produce chicks and improve their quality and future performance, which aims to the arrival of nutrients solutions to the embryo during the period of its embryonic development at the same time that the embryos perform feeding on the amnion fluid to take advantage of them either to drive growth or to increase the development of its internal organs and reduce embryonic mortality (Hamadani *et al.*, 2013). Authors have used many types of nutrients in different incubation periods all of them share one goal: increasing body weight, improving its future performance, speeding up the development of the gut and stimulating its ability to digest and absorb the newly hatched chicks. *In ovo* feeding also provides protection for the enteric mucosa during

**Author for correspondence* : E-mail: Alasady87@gmail.com

the first period of hatching, which is characterized by its sensitivity to pathogenic microbes due to the lack of levels of symbiotic microflora that grow and multiply in the mucous layer. Injecting some solutions such as sodium chloride, sucrose, maltose, dextrin, arginine, zinc-methionine have stimulated embryo growth and increased body weight when hatching and improved hatchability as well as the development and improvement of gut activity and stimulating the genetic expression of enzymes secreted from the intestine and nutrient transporters responsible for absorbing and transporting food (Obaid, 2010). Studies have gone into detail about *in ovo* injection of certain specific nutrients and their effect on the development of embryo and its internal organs. For example, the use of probiotics prevents disease-causing infections and injecting amino acids (L-arginine, L-lysine, L-histidine, threonine) are important in supporting growth performance as well as injecting vitamin C and E

improves immunity and that injecting carbohydrates increases glycogen storage, which promotes muscle growth (Peebles, 2018). Likewise, *in ovo* injection of sulfur-containing amino acids (methionine and cysteine) during embryonic development have positive effects on gene expression and antioxidant indicators as well as fat reduction for newly hatched chicks (Elnesr *et al.*, 2019). Many plants were characterized by a therapeutic and medicinal character and began to occupy a distinctive position in animal production due to their ability to stimulate the activity and growth of the main organs in the body. Many researchers used different nutrients for the embryo with embryonic ages and different injection sites *in ovo*. However, the use of plant seed extracts containing gum as early feeding for embryo has not been applied and there are no studies on it, with both methods of injection and immersion of hatching eggs. Examples of seeds containing gum are fenugreek seeds. It contains active compounds and gum in the composition, percentage of gum in it is 23%, sugars (galactomannan) as well as proteins, fats, fibers and carbohydrates with an estimated rate of 9.5, 10, 18.5 and 42.3%, respectively. As for their mineral elements and vitamins, they were calcium, phosphorus, potassium and vitamin A, B1, B2 and niacin was 1.3, 0.48, 1.7%, 1040 IU /100 g/ 0.41, 0.36 and 6 mg/100 g, respectively, as well as vitamin B6, saponins and flavonoids and small amounts of sucrose, glucose and fructose (Parthasarathy *et al.*, 2008; Raju and Rao, 2009). Use of fenugreek seeds as feed additives to increase the reproductive, physiological and productive performance of birds due to their content of effective active ingredients important for growth and plant hormones Weerasingha, Atapattu, (2013), Beghoul *et al.*, (2017). As for the use of oat, it was widely used in feeding poultry as a source of energy and protein in diets with the aim of increasing growth due to the presence of β -Glucan in its composition and constituting 3-5%, which is a soluble fiber due to its high viscosity (Anttila *et al.*, 2004). Also among the seeds of plants used in our study are basil seeds, it contains a gelatinous mass when soaked in water, which is a polysaccharides containing two parts, The first one is glucomannan, has a ratio of glucose to mannos (10:2), second part, it is similar to xylene, with a ratio of 43% and 24-29% respectively, from gum extract (Razavi *et al.*, 2009). We did not find the use of these extracts for embryos as nutrients and supportive materials during the embryonic period, so this study aims to show the effect of the immersion and injection methods of the aqueous extract of fenugreek, oat and basil seeds as early feeding for the embryos with the aim of increasing the hatchability and improving the post-hatching characteristics and quality of the hatched chicks.

Material and Methods

The study included the procedure of immersion of eggs before its introduction to the incubation machines for some treatments, other treatments which the eggs were injected with the aqueous extracts of fenugreek seeds, oats and basil at day 17 of the incubation to study their effect on the characteristics of hatching and the quality of hatched chicks. Work was carried out in the hatchery of Al-Anwar Poultry Company (Al-Mouradia District - Babylon Governorate) for the period from 5/4/2019 to 27/4/2019. A total of 1050 eggs broiler breeders (Ross-308) imported from the production of Al-Tahounah Agricultural Production Company (Jordan) and it was from one field, breeders aged of 34 weeks, The experiment treatments were designed as follows: Treatment T_C: Control group, treatments T_{DF}, T_{IF}: Included immersion and injection of hatching eggs with the aquatic extract of the fenugreek seeds, treatments T_{DO}, T_{IO}: Included immersion and injection of hatching eggs with the aquatic extract of the oat seeds, treatments T_{DB}, T_{IB}: Included immersion and injection of hatching eggs with the aquatic extract of the basil seeds. The concentration of all extracts was 6% of treatments', respectively. Perforated plastic baskets were used in which eggs were placed for each treatment very carefully in order to avoid any cracking in the shell and then immersed them in a larger plastic container than the baskets in which the prepared liquid is placed and its temperature is 25°C. As for the way of injection; It took place at the end 17th day in incubators and the injection was performed in the amniotic fluid after the eggs were scanned and at an angle of 45° deviation from the longitudinal axis in the wide part of the egg by using Zhu *et al.*, (2019) method, used a 23-gauge insulin syringe with a depth of 25 mm and a dose of 0.2 ml, close shell hole with nail dye. The eggs were weighed pre-incubation and then re-weighed before being transferred to hatching machines. The incubation and hatch time for each treatment was calculated by adding the number of hours from the beginning of the embryonic development (the time of egg acquisition to heat in the hatching machine) until the beginning of the hatching process (5% of the total fertilized eggs). As for the hatching window; According to the number of hours it takes for the chicks to complete the hatching process from the start of piped to the first chick until the exit of the last chick per treatment respectively, according to Tona *et al.*, (2005) and Nielsen *et al.*, (2010). Hatchability from fertilized eggs was calculated according to the method described by Al-Zjaji and Ibrahim, (1981). After the chicks hatch is complete weighed and calculated its vitality (Geidam *et al.*, 2007),

chick and shank length (Willemsen *et al.*, 2008) and wing length (Osaiyuwu *et al.*, 2009). The chicks were distributed for each treatment out of the total hatched chicks from 150 eggs and by 3 replicates per treatment according to the experiment design. Randomly distributed within pens, each pens is 1.5 × 2.5 m (30 birds per repetition). The chicks were breeding on a litter of sawdust (5cm) and spread cardboard paper over it starting from day 1-3 to ensure that the litter was not consumed and that digestion problems occurred for the first days of breeding. Used the continuous lighting program (24 hours / day) from the start of the experiment until the end of the fifth week of the bird's life. The chicks were fed on diets and included two diets: the first was a starter diet (crumble feed containing protein and energy of 22.3% and 3000 kcal) and used from the age of one day until the end of the third week. After that, it was replaced by the finisher diet (growth contains protein, 21.4% energy and 3100 kcal) until the end of the fifth week. Data were analyzed statistically by ANOVA using a completely randomized design (CRD). In case of significance difference, multiple range test was used (Duncn, 1955). Statistical software SAS, 2012 was used to carry out statistical analysis.

Results

Results in tables 1 and 2, indicate the effect of immersion and injection of hatching eggs in the aqueous extract of fenugreek, oat and basil seeds (T_{DF} , T_{DO} and T_{DB}) on the characteristics of hatching and hatched chicks

Table 1: Effect of immersion and injection of hatching eggs with aqueous extracts of fenugreek seeds, oats and basil on initial egg weight, relative water loss weight and hatchability of fertile and total eggs.

Treatments	Initial egg weight (g)	Egg weight at day 18 (g)	Relative water loss weight	Hatchability of fertile eggs (%)	Hatchability of total eggs (%)
T_C	62.91	56.41	10.31	94.45 ^a	91.27
T_{DF}	63.14	56.02	11.25	97.26 ^a	94.73
T_{IF}	62.65	56.08	10.45	91.74 ^a	89.90
T_{DO}	62.82	55.71	11.31	94.44 ^a	92.53
T_{IO}	61.70	55.33	11.28	93.04 ^a	90.50
T_{DB}	62.44	55.41	11.02	94.50 ^a	93.20
T_{IB}	62.45	56.20	10.00	76.30 ^b	72.73
SEM	0.552	0.400	0.781	1.411	1.642
<i>P</i> -value	0.7300	0.9791	0.8677	0.0005	0.0001

Experimental treatments: T_C : control treatment, T_{DF} , T_{IF} : treatments of immersion and injection of hatching eggs with aqueous extract of fenugreek seeds, T_{DO} , T_{IO} : treatments of immersion and injection of hatching eggs with aqueous extract of oats, T_{DB} , T_{IB} : treatments of immersion and injection of hatching eggs with aqueous extract of basil seeds. ^{a,b,c} Means in the same column bearing different superscripts differ significantly ($P < 0.05$) or (0.01). SEM = Standard error of means.

of broilers, table 1, shows that there was no significant effect on the characteristic of initial egg weight, egg weigh at day 18 between injection, immersion and control treatments, while a significant decrease ($P < 0.01$) occurred in hatchability from fertilized and total eggs for an basil injection treatment (T_{IB}) compared to the control treatment. While there were no significant differences between the treatments of immersion, other injections and control treatment in these two characteristics. The decrease in hatchability was 18.15 and 18.54% for the treatment of aquatic extract of basil seeds (T_{IB}) compared to the control treatment, respectively. Also, the Hatchability from fertilized eggs in the treatment of immersion with aqueous extract of fenugreek seeds (T_{DF}) has achieved the highest values between experimental treatments and increased arithmetical compared with the control treatment.

Table 2 shows a significant increase ($P < 0.01$) at incubation and hatch time for all immersion treatments and fenugreek and basil injection (T_{IF} and T_{IB}) compared to the control treatment, while no significant difference was shown between the oat injection treatment (T_{IO}) and the control treatment for above-mentioned characteristic and the number was Incubation and hatching hours 470.0, 473.0, 473.0, 474.0, 469.0, 474.0 and 498.0 hours for treatments T_C , T_{DF} , T_{IF} , T_{DO} , T_{IO} , T_{DB} and T_{IB} respectively. As the results indicated in the above table, the use of the immersion and injection method for hatching eggs with the aqueous extract of fenugreek seeds and immersion with oat and basil extract have achieved the best means

for the hatching window quality. All immersion and injection treatments except treatment of oat and basil aquatic extract (T_{IO} and T_{IB}) achieved a significant decrease ($P < 0.01$) in the general means of the hatching window characteristic when compared to control. The number of hatching window hours was significantly higher compared to control. Whereas, there was no significant difference in this characteristic between treatment of oat aqueous extract (T_{IO}) and control treatment. Fenugreek and basil immersion treatments (T_{DF} and T_{DB}) and oat injection treatment (T_{IO}) topped the best results with the highest average values between experimental treatments in the chick weight and chick weight/egg weight. Fenugreek and basil immersion treatments (T_{DF} and T_{DB}) and

Table 2: Effect of immersion and injection of hatching eggs with aqueous extracts of fenugreek seeds, oats and basil on hatching time, hatching window, chick weight and chick weight / egg weight.

Treatments	Hatching time (h)	Hatching window (h)	Chick weight (g)	Chick weight/ Egg weight
T _C	470.0 ^c	25.00 ^b	42.76 ^{cd}	67.96 ^{cd}
T _{DF}	473.0 ^b	20.00 ^c	45.22 ^b	71.68 ^b
T _{IF}	473.0 ^b	21.00 ^c	43.62 ^a	69.64 ^a
T _{DO}	474.0 ^b	20.00 ^c	44.34 ^a	70.58 ^a
T _{IO}	469.0 ^c	26.00 ^b	44.65 ^b	71.59 ^b
T _{DB}	474.0 ^b	17.00 ^d	47.79 ^a	75.61 ^a
T _{IB}	498.0 ^a	29.00 ^a	41.67 ^d	66.71 ^d
SEM	0.570	0.570	0.477	0.795
P-value	0.0001	0.0001	0.0001	0.0002

Experimental treatments: T_C: control treatment, T_{DF}, T_{IF}: treatments of immersion and injection of hatching eggs with aqueous extract of fenugreek seeds, T_{DO}, T_{IO}: treatments of immersion and injection of hatching eggs with aqueous extract of oats, T_{DB}, T_{IB}: treatments of immersion and injection of hatching eggs with aqueous extract of basil seeds. ^{a,b,c} Means in the same column bearing different superscripts differ significantly (P<0.05) or (0.01). SEM = Standard error of means.

oat injection treatment (T_{IO}) topped the best results with the highest average values between experimental treatments in the chick weight and chick weight/egg weight, as they all significantly (P<0.01) compared to the control treatment, where the basil immersion treatment (T_{DB}) recorded the highest values between the treatments and the chick weight for this treatment was 47.97 g while it was 42.76 g for control treatment, It was followed by an increase in the field of immersion with fenugreek extract (T_{DF}), which had a general average of 45.22 g.

Table 3: Effect of immersion and injection of hatching eggs with aqueous extracts of fenugreek seeds, oats and basil on vitality, chick length, shank length, Tarsometatarsus Diameter and wing length.

Treatments	Vitality (sec)	Chick length (cm)	Shank length (cm)	Tarsometatarsus diameter (mm)	Wing length (cm)
T _C	4.80 ^a	17.12 ^b	2.81 ^{bc}	3.32 ^{ab}	3.40
T _{DF}	4.80 ^a	17.50 ^{ab}	2.91 ^{ab}	4.46 ^a	3.35
T _{IF}	4.73 ^a	17.34 ^{ab}	2.90 ^{ab}	3.45 ^a	3.39
T _{DO}	3.96 ^a	17.42 ^{ab}	2.93 ^a	3.45 ^a	3.52
T _{IO}	4.66 ^a	17.34 ^{ab}	2.93 ^a	3.45 ^a	3.54
T _{DB}	4.53 ^a	17.74 ^a	2.94 ^a	3.49 ^a	3.34
T _{IB}	8.73 ^b	16.68 ^c	2.78 ^c	3.18 ^b	3.49
SEM	0.557	0.115	0.234	0.057	0.075
P-value	0.0066	0.0020	0.0228	0.0500	0.4815

Experimental treatments: T_C: control treatment, T_{DF}, T_{IF}: treatments of immersion and injection of hatching eggs with aqueous extract of fenugreek seeds, T_{DO}, T_{IO}: treatments of immersion and injection of hatching eggs with aqueous extract of oats, T_{DB}, T_{IB}: treatments of immersion and injection of hatching eggs with aqueous extract of basil seeds. ^{a,b,c} Means in the same column bearing different superscripts differ significantly (P<0.05) or (0.01). SEM = Standard error of means.

While there were no significant differences between the treatments of fenugreek, basil and oat immersion (T_{IF}, T_{IB} and T_{DO}) and control treatment in these two characteristics.

Table 3, shows the effect of immersion and injection of hatching eggs with aqueous extracts of fenugreek, oat and basil seeds in the characteristics of the hatched chicks, as we note a significant decrease (P<0.01) in agility of the hatched chicks for the treatment of injection with the aqueous extract of basil seeds (T_{IB}) compared to the control treatment, while no significant differences were observed between the other treatments of immersion and injection with control treatment. As for length of the chick, the basil immersion treatment (T_{DB}) was unique by having the highest average values for experimental treatments, it was significantly different (P<0.01) compared to control and basil injection (T_{IB}) treatments, as well as significant differences between control treatment and basil injection treatment. Whereas, no significant difference appeared between control treatment and immersion and injection treatments of fenugreek and oat extract (T_{DF}, T_{IF} and T_{IO}). The results from the same table indicate a significant effect (P<0.05) on the shank length, Where the treatments of immersion oat and basil and oat injection were superior to the control treatments and basil injection and no significant effect was observed between the control and fenugreek immersion treatment and fenugreek and basil injection. Whereas, no significant difference appeared between control treatment and immersion and injection treatments of fenugreek and oat extract (T_{DF}, T_{IF} and T_{IO}).

As for the characteristic tarsometatarsus diameter; Basil injection treatment (T_{IB}) showed the lowest values for general means and decreased significantly (P<0.05) compared to the parameters for fenugreek, oat, basil immersion, fenugreek and oat injection (T_{DF}, T_{DO}, T_{DB}, T_{IF}, T_{IO}) and that these treatments did not show significant differences with the control treatment. The above table did not indicate any significant differences between control treatment and immersion and injection treatments in the wing length characteristic.

Table 4, indicates the effect of immersion and injection of hatching eggs with aqueous extracts of fenugreek, oat and basil seeds on the characteristic of embryonic mortality, the absence of significant differences between treatment of control and treatments of

Table 4: Effect of immersion and injection of hatching eggs with aqueous extracts of fenugreek seeds, oats and basil on embryonic mortality, Pipped eggs and total embryonic mortality.

Treatments	Embryonic mortality (%)			Piped eggs (%)	Total embryonic mortality (%)
	Early Days 0-7	Middle Days 8-14	Late Days 15 to hatch		
T _C	1.51	0.00	2.79 ^b	0.61 ^{ab}	4.16 ^b
T _{DF}	1.38	0.00	0.68 ^b	0.00 ^b	2.06 ^b
T _{IF}	2.06	1.38	3.39 ^b	0.68 ^{ab}	6.85 ^b
T _{DO}	2.06	1.98	1.51 ^b	0.00 ^b	5.54 ^b
T _{IO}	2.87	1.32	0.77 ^b	1.93 ^{ab}	4.96 ^b
T _{DB}	2.11	0.68	0.75 ^b	1.45 ^{ab}	3.55 ^b
T _{IB}	2.16	1.46	11.59 ^a	2.88 ^a	15.21 ^a
SEM	0.705	0.491	1.272	0.567	1.458
P-value	0.8984	0.2626	0.0038	0.0854	0.0039

Experimental treatments: T_C: control treatment, T_{DF}, T_{IF}: treatments of immersion and injection of hatching eggs with aqueous extract of fenugreek seeds, T_{DO}, T_{IO}: treatments of immersion and injection of hatching eggs with aqueous extract of oats, T_{DB}, T_{IB}: treatments of immersion and injection of hatching eggs with aqueous extract of basil seeds. ^{a,b,c} Means in the same column bearing different superscripts differ significantly (P<0.05) or (0.01). SEM = Standard error of means.

immersion and injection in the characteristic of embryonic mortality in the early and middle period of incubation. While it was found that there was a significant increase (P<0.01) in the general means of mortality in the late stage and total embryonic mortality for the treatment of basil injection compared to the control treatment and other immersion and injection treatments, as it reached 8.80 and 11.05% for the characteristics the mentioned treatment is compared to control. As for the characteristics of piped eggs, no significant differences were observed between treatment of control and treatments of immersion and injection of aqueous extracts of fenugreek, oat and basil seeds. Whereas, there was a significant increase (P<0.05) in the treatment of basil extract (T_{IB}) injection compared to the two treatments of fenugreek and oat extract (T_{DF} and T_{DO}). As for live piped chick percentage, no significant differences were observed between treatment of control, immersion and injection with aqueous extracts of fenugreek, oat and basil seeds.

Discussion

Many authors have endeavored to raise the means of hatchability with the aim of increasing profits and reducing the embryonic mortality of commercial hatchery owners, they used materials and elements to *in ovo* during embryonic development, of them, they have achieved the desired goal and some of them have failed. Where Lahav *et al.*, (2012) tested the efficacy of 44 substances, including polymers, polysaccharides, oils, proteins, plants gum, natural resins, carbon compounds and natural tree sap, where immersion was applied to eggs hatching broiler with the aim of increasing

hatchability. Hajati *et al.*, (2014) studied injecting 4.5 mg of grape seed extract on day 18 of the incubation and obtained significant results for increasing hatchability, In the same context, Shahein and Sedeek, (2014), when spraying the hatching eggs for Gimmizah chicken with Propolis, reached a level of 7 and 14% that raised the percentage of hatchability from fertilized eggs: 85.62% for the control treatment to 96.58 and 95.76% for the mentioned levels, respectively. As for Nanle *et al.*, (2017), it achieved distinctive results when it studied the effect of injecting hatching eggs with *Moringa oleifera* leaf extract and concluded that the treatment was 0.5 µg with the highest incidence of hatchability compared to other treatments. As for our current study; We have demonstrated

that some plant seed extracts (immersion fenugreek seeds) have increased hatchability arithmetical and are due to the fact that their aqueous extract contains a high percentage of linolenic acid, which about 49-75% of the oil content in the seeds of 15.9% (Mathews *et al.*, 1993; Nour *et al.*, 2009), this was confirmed by Amen, (2016) to improve hatchability 86.31 to 90.14% when injected *in ovo*. Or perhaps it is because its aqueous extract contains a wide spectrum of amino acids that have been shown to be effective in raising hatchability (Bhanja *et al.*, 2014; Kita *et al.*, 2015). We have emerged some extracts (the injection of basil seeds) that when delivered to the embryo, problems occur during embryonic development, which may continue to affect future performance. As the arrival of the aqueous extract of basil seeds to embryo by injection method reduced the hatchability by 18.15 and 18.54% for fertilized and total eggs respectively when compared to the control treatment. The reason for the significant decrease is that basil seeds contain saponins such as apigenin (Charles, 2013). That have been shown to be able to analyze red blood cells (Hemolysis), or that they affect increased permeability of membranes (Abdul Jalil, 2014), Consequently, their presence with the nutritional content of the egg causes a disruption of the overall biological system of embryo growth. Or as a result of the basil seed extract contains some of the compounds (Eugenol, Ursolic acid and Carvacrol) that appear to prevent cell growth *in vitro* and induce them to apoptosis, explained by Shah *et al.*, (2015) When they found in their study that the alcoholic injection of basil leaves into hatching eggs at exactly 24, 48 and 72 hours after the sixth day of

embryonic development of chicken, a negative effect on embryo growth appeared, the number of blood vessels decreased significantly and the area of the chorioallantoic membrane (CAM) decreased. This is an explanation that may lead us to the reason why the above attribute is low in the results of our study. However, when the same substance is present on the shell by immersion in the eggs, it is possible to remove the bad effect of these compounds present in the basil seeds that may be believed to be completed by the filtering of the shell membranes when used by the embryo in conjunction with the calcification process, which occurs and is complete at day 13 of embryo development. (Al-Zajaji and Ibrahim, 1981).

It is a note of the adjective time of incubation and hatching; The reason for the increase in the number of hours and the delay in hatching time in some immersion treatments may be the formation of a layer of gums on the egg shell before it is inserted into the incubation machines, as it leads to a delay in the time of acquiring heat *in ovo* contents, which were inconsistent with the findings of Fouad, Abdel-Hafez, (2017), Fouad *et al.*, (2019, 2018) when they sprayed quail hatching eggs with live yeast, garlic oil and sprinkled Dandarawi chicken hatching eggs with vinegar, which reduced the number of hours of incubation and hatching significantly, while the increase in the number of incubation and hatching hours in some injection treatments may be due to the delay at the time of injection when the eggs are released outside the incubator on day 17th of embryonic development, these results were consistent with what was found by Mohammed *et al.*, (2011) who indicated when the eggs in the incubator were cooled to a degree The temperature of 24°C and for 6 hours a day 16 days of incubation led to an increase in the number of hours of incubation and hatching about 8.45 hours, hatching time is a very important sign of hatcheries, to avoid chicks stay longer and expose it to dehydration.

The effect of applying immersion and injection methods on plant seed extracts was evident in the characteristic of the hatching window. The reason for the decrease in the number of hatching window hours in most treatments of immersion and injection may be due to an increase in the activity and vitality of the chicks, which are the result of an increase in the accumulation of liver glycogen stores in the last period of hatching which gives the chick an energy that enables him to make the hatching process faster which was mentioned by Moran, (2007) and Obaid, (2010). The seeds used contain the essential amino acids that form glycogen by creating glucose through the gluconeogenesis process, the fact that oxygen is limited during the last quarter of incubation

period and promote embryo growth and reduce the duration of hatching (Bhanja and Mandal, 2005; Gao *et al.*, 2017). Or due to the seeds used (fenugreek seeds) contain glucose-containing compounds such as G-C6- α -galactosyl 3-Sucrose 6 and digalactosyl myo-inositol (Leela and Shafeekh, 2008), that helps in the hatching process and this is a possible explanation for the low number of hatching window hours. Immersion and some injection treatments resulted in an increase weight of chicks and the ratio of the chicks weight to egg weight, this increase may be due to the aqueous extract of the seeds used to contain polysaccharides that increase accumulation of glycogen in the liver and muscles before hatching (Kornasio *et al.*, 2011), or to stimulate the evolution and division of satellite cells. Moore *et al.*, (2005) explained that cell activity begins before hatching and reaches its highest rate shortly after hatching and then begins to decrease gradually after seven days. Or perhaps it is due to the maintenance of protein in the pectoral muscles during hatching. Our study agreed with El-Hanoun and Mossad, (2008), who concluded that baking duck eggs with paraffin wax on day 14 of the incubation raised the weight of the resulting ducklings from 45.4 to 50.7 g. And with Salmanzadeh *et al.*, (2012) who worked to arrival glucose and magnesium *in ovo* -white area and on the 17 day of incubation and obtained a significant increase in the weight of the hatched chicks. And with Fouad and Abdel-Hafez, (2017) who used the spray method to deliver live yeast on quail eggs. And with Fouad *et al.*, (2019) when using vinegar spray on eggs with a concentration of 2.5%, the weight of the chicks increased by 2.5 g compared to the control. It was inconsistent with Bhanja *et al.*, (2008) who observed that there was no significant effect on the weight of the incubated chicks when injected with carbohydrates. We did not find any studies that support this study of the effect of immersion or injection of hatching eggs with plant extracts containing gums (fenugreek, oats and basil seed) in embryonic growth, hatching characteristics and weight of chicks hatched.

The injection of aqueous extract of basil seeds reduced the vitality hatched chicks as a result of an increase in the incubation and hatching period and the number of hours needed to complete the hatching process, which reached 29 hours and exhausted the energy spent on this process and thus negatively affected its vitality and exit, weak and exhausted. Or, the reason may be due to a decrease in the level of energy available in the body after hatching due to an optimal decrease in the use of yolk, which is reflected in its activity and vitality. Among the reasons that led to an increase in chick length, length and shank diameter in some immersion and injections

treatments are the increase in the formation of nutrients during the period of embryonic development and benefit from them that are carried in the aqueous extract containing the gums. Where studies indicated a direct relationship between the chick's length and productive performance (Willemsen *et al.*, 2008; Michalczuk *et al.*, 2011). The chick length as an indication is better than chick weight to predict future performance due to the presence of the remaining non-absorbed yolk that is calculated with chick weight (Mukhtar *et al.*, 2013) on the one hand and between leg length and productive performance on the other hand (Wolanski *et al.*, 2006). Patbandha *et al.*, (2017) concluded that each centimeter of chick length gives a future weight gain of 113 - 214g at day 43 of the breeding. These results were consistent with what Fouad and Abdel-Hafez, (2017) observed when spraying quail hatching eggs with live yeast had significantly increased chick length from 9.23 to 10.03 cm. The reason for shank length may be due to the fenugreek and basil seeds containing a high amount of calcium and magnesium (Muhammad and Abdel-Elah, 2009), it was confirmed by the results obtained by Ghobadi and Hemati Matin, (2015), who concluded that injecting different levels of calcium enabled them to accelerate the growth, maturity and development of bone cells.

The application of immersion and injection did not affect embryonic mortality. While the arrival of the fenugreek and basil seed extract method by immersion reduced a total embryonic mortality, which may be due to their aqueous extract containing polysaccharides that give the embryo ability to cause hatching process, which appeared and was reflected in the decrease in the number of hours needed to complete the hatching process (hatching window), except for the treatment of aqueous extract of basil seeds, Which may be due to its effect on the analysis of erythrocytes or affect the permeability of membranes and gas exchange, which causes a problems in the general biological system, which has been evident by the high percentage of piped eggs (Abdul Jalil, 2014) and it may also be due to not using the remaining highly efficient yolk, which is the only direct available source of energy (Lambson, 1970). Thus, it may cause low energy sources and an inability to hatch. Shah *et al.*, (2015) confirmed it to chicken embryos and Gamallo *et al.*, (2016) confirmed it to duck embryos.

Conclusions

In this research work, the application of the immersion method for hatching eggs with the aqueous extract of fenugreek and basil seeds has improved most of the characteristics of hatching and hatched chicks for broilers.

References

- Abdel Jalil, S. A-A.M. (2014). Plant natural products. Alexandria University. First edition. Garden of Knowledge Library - Egypt.
- Al-Zajaji, R.J and I.K. Ibrahim (1982). Hatching and hatchery management. Ministry of Education and Scientific Research. Directorate of Dar Al-Kitab for Printing and Publishing - University of Mosul. First edition.
- Amen, M.H.M. (2016). *In ovo* feeding of omega-3 fatty acids improved production traits, haematological parameters and immune response in broiler. *Res. Opin. Anim. Vet. Sci.*, **6(1)**: 1-6.
- Anttila, H., T. Sontag-Strohm and H. Salovaara (2004). Viscosity of beta-glucan in oat products. *Agric. Food. Sci.*, **13**: 80-87.
- Beghoul, S., M.C. Abdeldjelil, H. Benazzouz, A. Messai, B. Chouah and A. Mansour (2017). Fenugreek (*Trigonella foenum-graecum*): An alternative antistress in broiler chickens in Algeria. *Der Pharmacia Lettre.*, **9(1)**: 64-69.
- Bhanja, S.K and A.B. Mandal (2005). Effect of *in ovo* injection of critical amino acids on pre and post-hatch growth, immunocompetence and development of digestive organs in broiler chickens. *Asian Aust. J. Anim. Sci.*, **18(4)**: 524-531.
- Bhanja, S.K., A.B. Mandal, S.K. Agarwal and S. Majumdar (2008). Effect of *in ovo* glucose injection on the post hatch-growth, digestive organ development and blood biochemical profiles in broiler chickens. *Indian J. Anim. Sci.*, **78**: 869-872.
- Bhanja, S.K., M. Sudhagar, A. Goel, N. Pandey, M. Mehra, S.K. Agarwal and A. Mandal (2014). Differential expression of growth and immunity related genes influenced by *in ovo* supplementation of amino acids in broiler chickens. *Czech J. Anim. Sci.*, **59(9)**: 399-408.
- Charles, D.J. (2013). Antioxidant properties of spices, herbs and other sources. chapter 11 basil. 173-179. Springer New York Heidelberg Dordrecht London.
- Duncan, D.B. (1955). Multiple range and multiple test. *Biometris.*, **11**: 1-42.
- El-Hanoun, A.M and N.A. Mossad (2008). Hatchability improvement of Peking duck eggs by controlling water evaporation rate from the egg shell. *Egypt Poult. Sci.*, **28(2)**: 767-784.
- Elnesr, S.S., H.A.M. Elwan, Q.Q. Xu, C. Xie, X.Y. Dong and X. T. Zou (2019). Effects of *in ovo* injection of sulfur containing amino acids on heat shock protein 70, Corticosterone hormone, antioxidant indices and lipid profile of newly hatched broiler chicks exposed to heat stress during incubation. *Poult. Sci.*, 609-615.
- Fouad, W. and M.S. Abdel-Hafez (2017). Effect of spraying hatching eggs of Japanese quail by live yeast on physiological changes in the embryonic development, hatchability and total bacterial count. *Egypt. Poult. Sci.*, **37(3)**: 1303-1321.

- Fouad, W., M.S. Abdel-Hafez and H.A.H. Abd El-Halim (2018). Influence of spraying garlic oil on embryonic development, hatchability, physiological parameters, post-hatch chick growth and bacterial contamination of fertile quail eggs. *Egypt. Poult. Sci.*, **38(3)**: 877-893.
- Fouad, W., M.G. Abdelfattah and M.A. Abdelnabi (2019). Effect of spraying hatching eggs by different levels of vinegar on embryological development, hatchability and physiological performance of Dandarwi chicks. *Egypt. Poult. Sci.*, **39(1)**: 291-309.
- Gamallo, J.P.M., G. Espere, D.M. Carillo, D.N. Blanes, F.G. Abuda, H.J. Labarda, X.M. Madelo and J.C. Jumawan (2016). Evaluation of antiangiogenic property of *Ocimum basilica* ethanolic leaf extract by using duck embryo chorioallantoic membrane (CAM) assay and its morphometric analysis. *International Journal of Herbal Medicine.*, **4(4)**: 22-26.
- Gao, T., M.M. Zhao, Y. J. Li, L. Zhang, J.L. Li, L.L. Yu, F. Gao and G.H. Zhou (2017). Effects of *in ovo* feeding of L-arginine on the development of digestive organs, intestinal function and post-hatch performance of broiler embryos and hatchlings. *J. Anim. Physiol. Anim. Nutr.*, **102**: e166-e175.
- Geidam, Y.A., U.I. Ibrahim, M.M. Bukar, H.I. Gambo and O. Ojo (2007). Quality assessment of broiler day-old chicks supplied to Maiduguri. North-Eastern Nigeria. *Int. J. Poult. Sci.*, **6**: 107-110.
- Ghobadi, N. and H.R. Hemati Matin (2015). Response of broiler chicks to *in ovo* injection of calcium, phosphorus and vitamin D complex (CaDPhos). *Global Journal of Animal Scientific Research.*, **3(2)**: 536-543.
- Hajati, H., A. Hassanabadi, A. Golian, H. Nassiri-Moghaddam and M.R. Nassiri (2014). The effect of *in ovo* injection of grape seed extract and vitamin C on hatchability, antioxidant activity, yolk sac absorption, performance and ileal micro flora of broiler chickens. *Res. Opin. Anim. Vet. Sci.*, **4(12)**: 633-638.
- Hamadani, H., A. Khan, M. Banday and A. Hamadani (2013). Early chick feeding and *in-ovo* nutrition - two managemental strategies to combat the effects of delayed feeding. *Int. J. Modern Plant and Anim. Sci.*, **1(3)**: 123-141.
- Kita, K., K.R. Ito, M. Sugahara, M. Kobayashi, R. Makino, N. Takahashi, H. Nakahara, K. Takahashi and M. Nishimuka (2015). Effect of *in ovo* administration of branched-chain amino acids on embryo growth and hatching time of chickens. *J. Poult. Sci.*, **52**: 34-36.
- Kornasio, R., O. Halevy, O. Kedar and Z. Uni (2011). Effect of *in ovo* feeding and its interaction with timing of first feed on glycogen reserves, muscle growth and body weight. *Poult. Sci.*, **90(7)**: 1467-1477.
- Lahav, J., P. Carmiel and N. Kibbutz El-Rom (2012). Methods and composition for increasing the hatchability of hatchery eggs. Patent No.: US 8,327,798 B2. Dec. 11.
- Lambson, R.O. (1970). An electron microscopic study of the endodermal cells of the yolk sac of the chick during incubation and after hatching. *Am. J. Anat.*, **129**: 1-20.
- Leela, N.K and K.M. Shafeekh (2008). Fenugreek. In: Chemistry of Spices (eds. V.A. Parthasarathy, B. Chempakam and T.J. Zachariah). Biddles Ltd, UK, 242-259.
- Mathews, S., R.S. Singhal and P.R. Kulkarni (1993). *Ocimum basilicum*: A new non-conventional source of fiber. *Food Chemistry.*, **47**: 399-401.
- Michalczuk, M., M. Stepinska and M. Lukasiewicz (2011). Effect of the initial body weight of Ross 308 chicken broilers on the rate of growth. *Annals of Warsaw University of Life Sciences - SGGW Animal Science.*, **49**: 121-125.
- Mohammad, W.Y and S.M. Abdel-Elah (2009). Isolate the active substances in *Trigonella foenum-graecum* seeds and study their vital effectiveness. *Anbar University Journal of Pure Sciences.*, **3(3)**: 1-10.
- Mohammed, K.A., A. El-Boghdady, M.A.H. Soliman, M.A. Abd Al-Galil and N.M. AbdAl-Aleem (2011). The effect of both pre-incubation dipping eggs in vitamin C and cooling eggs during incubation period on embryonic and hatchability parameters two local chicken strains. *Egypt. Poult. Sci.*, **31(2)**: 379-392.
- Moore, D.T., P.R. Ferket and P.E. Mozdziaik (2005). The effect of early nutrition on satellite cell dynamics in the young turkey. *Poult. Sci.*, **84**: 748-756.
- Moran, E.T. (2007). Nutrition of the developing embryo and hatchling. *Poult. Sci.*, **86**: 1043-1049.
- Mukhtar, N., S.H. Khan and M. Anjum (2013). Hatchling length is a potential chick quality parameter in meat type chickens. *World's Poultry Science Journal.*, **69(4)**: 889-896.
- Nanle, O.N., A. Tété-Bénissan, K. Tona, A. Tete, K. Voemesse, E. Decuyper and M. Gbeassor (2017). Effect of *in ovo* inoculation of *Moringa oleifera* leaves extract on hatchability and chicken growth performance. *Europ. Poult. Sci.*, **81**: <https://www.researchgate.net/publication/323809510>.
- Nielsen, B.L., H.R. Juul-Madsen, S. Steinfeldt, J.B. Kjaer and P. Sorensen (2010). Feeding activity in groups of newly hatched broiler chicks: Effects of strain and hatching time. *Poult. Sci.*, **89**: 1336-1344.
- Nour, A.H., S.A. Elhoussein, N.A. Osman and H.A. Nour (2009). Characterization and chemical composition of the fixed oil of fourteen basil (*Ocimum basilicum* L.) accessions grown in sudan. *International Journal of Chemical Technology.*, **1(2)**: 52-58.
- Obaid, T.A. (2010). Biology and hatching technology in poultry. Faculty of Agriculture - Kafir El-Sheikh University. First edition. Knowledge facility in Alexandria.
- Osaiyuwu, O.H., A.E. Salako and O. Adurogbangban (2009). Body dimensions of Fulani and Yoruba ecotype chickens under intensive systems of management. *J. of Agri. Forestry and the Social Sciences (JOAFSS).*, **(7)2**: 195-201.
- Parthasarathy, V.A., B. Chempakam and T.J. Zachariah (2008). Chemistry of Spices. CABI International, London.
- Patbandha, T.K., D.D. Garg, S. Marandi, D.G. Vaghamashi, S.S.

- Patil and H.H. Savsani (2017). Effect of chick weight and morphometric traits on growth performance of coloured broiler chicken. *Journal of Entomology and Zoology Studies.*, **5(6)**: 1278-1281.
- Peebles, E.D. (2018). *In ovo* applications in poultry: A review. *Poult. Sci.*, 1-17. <https://www.ncbi.nlm.nih.gov/pubmed/29617899>.
- Raju, J and C.V. Rao (2009). Fenugreek (Diosgenin) In: Aggarwal, B.B. and A.B. Kunnumakkara. Molecular targets and therapeutic uses of spices: Modern uses for ancient medicine. Singapore: *World Scientific.*, 173-196.
- Razavi, S., S. Mortazavi, L. Matia-Merino, S. Hosseini-Parvar, A. Motamedzadegan and E. Khanipour (2009). Optimisation study of gum extraction from basil seeds (*Ocimum basilicum* L.). *International Journal of Food Science and Technology.*, **44**: 1755-1762.
- Salmanzadeh, M., Y. Ebrahimnezhad, H.A. Shahryar and R. Beheshti (2012). The effects of *in ovo* injection of glucose and magnesium in broiler breeder eggs on hatching traits, performance, carcass characteristics and blood parameters of broiler chickens. *Arch. Geflügelk.*, **76**: 277-284.
- SAS (2012). SAS user's guide : statistical system, Inc. Cary NC. U.S.A.
- Shah, U.H., G.R. Gonjari and A.E. Patil (2015). Influence evaluation of *Ocimum Sanctum* leaf extract on angiogenesis by using chick chorioallantoic membrane (CAM) assay. *Global Journal of Science Frontier Research: C Biological Science.*, **15(4)**: 13-20.
- Shahein, E.H.A and E.K. Sedeek (2014). Role of spraying hatching eggs with natural disinfectants on hatching characteristics and eggshell bacterial counts. *Egypt. Poult. Sci.*, **34(1)**: 213-230.
- Tona, K., B. Kemps, V. Bruggeman, F. Bamelis, L.D. Smit, O. Onagbesan, J.D. Baerdemaeker and E. Decuyper (2005). Comparison of three lines of broiler breeders differing in ascites susceptibility or growth rate. 1. Relationship between acoustic resonance data and embryonic or hatching parameters. *Poult. Sci.*, **84**: 1439-1445.
- Weerasingha, A.S and N.S.B.M. Atapattu (2013). Effects of fenugreek (*Trigonella foenum-graecum* L.) seed powder on growth performance, visceral organ weight, serum cholesterol levels and the nitrogen retention of broiler chicken. *Trop. Agric. Res.*, **24**: 289-295.
- Willemsen, H., N. Everaert, A. Witters, L. De Smit, M. Debonne, F. Verschuere, P. Garain, D. Berckmans, E. Decuyper and V. Bruggeman (2008). Critical assessment of chick quality measurements as an indicator of posthatch performance. *Poult. Sci.*, **87**: 2358-2366.
- Wolanski, N.J., R.A. Renema, F.E. Robinson, V.L. Carney and B.I. Fancher (2006). Relationship between chick conformation and quality measures with early growth traits in males of eight selected pure or commercial broiler breeder strains. *Poult. Sci.*, **85(8)**: 1490-1497.
- Zhu, Y.F., S.Z. Li, Q.Z. Sun and X.J. Yang (2019). Effect of *In Ovo* feeding of vitamin C on anti oxidation and immune function of broiler chickens. *Animal.*, 1-7.