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THE EFFECT OF IN-OVO INJECTION OF SOME NUTRIENTS ON PRODUCTIVE PERFORMANCE AND SOME PHYSIOLOGICAL TRAITS OF HUBBARD BROILER CHICKS

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ABSTRACT: In total, 864 fertile eggs from Hubbard breeder hens were obtained from a commercial hatchery and were randomly distributed in a factorial arrangement of 3*3, factorial design with three levels of L-Arginine (L-Arg) (0%, 1% and 2%) and three levels of fresh Royal Jelly (RJ) (0%, 10% and 20%), in nine treatments containing (96 eggs each). Results showed that in ovo injection with 1, 2% L-Arg, or 10% RJ improved hatchability by 6.25, 7.29 and 8.33%, respectively, compared with those of non-injected treatment. Whereas, injection of 20% RJ insignificantly decreased hatchability percentage compared with non-injected group. The in ovo injection of eggs with 1, 2% L-Arg, or 10 and 20% RJ improved chicks' weight at hatch by 2.34, 1.63, 1.65 and 3.91%, respectively compared with those of non-injected group. Also, injection of eggs by 1, 2% L-Arg or 10 and 20% RJ produced chicks have higher relative intestine weight by 0.3, 1.34, 0.64 and 0.16%, respectively over those of non-injected group. However, chicks injected with the mixture of 2% L-Arg and 10% RJ recorded reduction in the relative intestine weight (-0.28%) among the other mixtures, compared with the results of non-injected group. Results indicated that the in ovo injection of L-Arg and RJ was effective in increasing the liver glycogen content of Hubbard chicks at hatch, and this trend increase with the increase of level of nutrient and with mixing between both nutrients. Results showed that the treatment levels whatever L-Arg or RJ has insignificant influence on villi width, length and surface area. Furthermore, the injection of both 1 and 2% of L-Arg were more effective than other levels 10 and 20% or RJ levels with respect of villi width. Results indicated that the highest relative sodium glucose co-transporter 1 gene expression value produced with Hubbard broiler breeder eggs injected with the studied low levels of L-Arg and RJ. In conclusion, it can be concluded that the in ovo injection with 10% RJ and 2% Arg may be recommend to apply with Hubbard breeder eggs to achieve good hatching traits. Also, the in ovo injection with 1% Arg+20 % RJ mixture has good improvement on intestine weight and villi measurements (gut development) at hatch.

Key words: In ovo injection-L-Arg,RJ-Hatchability-liver glycogen and SGLT1 gene expression.

INTRODUCTION

The in ovo injection of nutrients offers promise of sustaining the progress in production efficiency of commercial poultry (Stephanie Roto et al. 2016). The chick's first meal occurs when it imbibes the amnion prior to hatch, and so this is the first opportunity for nutritional programming (Ferket, 2014). Newly, the 21-day incubation period and the 10-day post-hatch period of the chick make up about 50% of the weight of chicks at market size. Therefore, incubation and embryonic development towards hatch is becoming of more relative importance to the successful rearing of meat poultry than ever before (Foye et al., 2007).

Above all, in ovo injection of nutrients has increased significantly hatchling weights by 3% to 7% over controls, and this advantage is often sustained at least until 14 days post-hatch. In addition to the increased body weights typically observed at hatch, the positive effects of in ovo injection of nutrients may include increased hatchability (Uni et al., 2005); enhanced gene expression of nutrient transporters, SGLT-1 (Foye et al., 2007); increased liver glycogen status (Foye et al., 2006 a, b); enhanced feed intake initiation behavior (De Oliveira et al., 2007).

Arginine is considered to be an essential amino acid in birds, particularly in the starter phase of development after hatching. Birds are incapable of synthesizing Arg because the urea biochemical cycle is not functional. Birds have the highest requirement of Arg compared with other animals (Ball et al., 2007). Al-Asadi (2013) reported that in ovo administration of Arg and lysine at 18 days of age increased hatchability, chick weight at hatch and at 42 days of age, as well as feed intake of broiler chickens.

Royal Jelly is a functional food secreted by the hypopharyngeal and mandibular glands of worker honeybees for the sole nourishment of the Queen bee (Drapeau et al., 2006). Moghaddam et al. (2014) found that in ovo injection of RJ into both sacs increased chicks' absolute and relative body, weight compared to the control.

The aim of this study is to evaluate the effects of in-ovo injection of three levels of L-Arg (0%, 1% and 2%) and three levels of fresh RJ (0%, 10% and 20%), or their mixtures on productive, some physiological traits and relative sodium glucose co-transporter 1 gene expression of Hubbard broiler hatchlings.

MATERIALS AND METHODS.

Eggs incubation and experimental design: A total number of 864 fertile eggs were collected from Hubbard breeder hens which were obtained from a commercial hatchery (Cairo company for Hatchers, Sadat City, Egypt) and incubated under optimal conditions at the Poultry Research Center, Faculty of Agriculture, Alexandria University. Before setting, eggs were weighed and divided into 9 experimental groups with nearly equal weight (each treatment has four replicates, with 24 eggs in each one). Fertile eggs were set in an electric forced draft incubator for first 18 days of incubation under 37.8 ^{o}C and 53± 3% relative humidity. The eggs were turned automatically 24 times per day with angles ±45^{o}, after that were removed to the hatcher, in covered trays after injection process, for the last three days of incubation under 37.5 ^{o}C and 70 ± 3 % relative humidity. Throughout the incubation period, all eggs were incubated according to the common routine procedures. On the day 21, the hatched chicks were weighed and transferred to grow-out house.

In Ovo Injection Solutions: The in ovo injection solutions of the present study include three levels (0, 1 and 2%) of L-Arg (L-Arg Monohydrochloride ≥98.5 %) produced by Ph. Eur., USP via Carl Roth, Germany. Also, three levels (0, 10 and 20%) of fresh RJ produced by Qingdao Fraken International Trading Co., Ltd, China. The chemical composition of the fresh RJ was; 63.1, 15, 7, and 1.2%, moisture, protein, total sugar and ash, respectively.

In ovo injection procedures: at 18-day of incubation, the eggs were candled and those with evidence of living embryos were transferred to the hatcher. All the eggs were taken out of the incubator for nearly 20 min/ tray to equalize the conditions for the injection process for all treatment groups. Eggs were candled for embryo examination of amniotic fluid positions, and to distinguish the proper injection site. A mini grinder was used to make a proper hole on the egg shell. Using 21 needle-gauge at 432h (18 d of incubation), eggs from all injection groups were injected from the top of the egg (upper side which closest to the amniotic fluid site) with in ovo injection solutions (0.5 ml/ egg) into the amniotic fluid as described by Uni and Ferket (2003) and Uni et al. (2005). After injection, the site of injection was sanitized with ethanol 70 % and sealed by using the wax gun. In parallel, the non-injected group was taken out of the incubator and kept in the same environmental conditions during treatments.

Measurements: Hatchability percentage of fertile egg (Scientific Hatchability % = number of chicks/ numbers of fertile eggs * 100) was calculated in each replicate at the end of incubation period. The hatchlings weight in grams was detected by weighing the chicks individually immediately after hatch. Plasma total glucose was determined by an enzymatic colorimetric test by specific diagnostic kits according to Trinder (1969). Intestine (jejunal) samples were taken at hatch and fixed in 10% neutral buffered formalin solution, dehydrated in ascending grades of ethanol, cleared in xylene and then embedded in paraffin wax and then blocked. Tissue sections of 5-6 Microns thick were made from the paraffin blocks and stained with hematoxylin and eosin (H&E) according to the method of Bancroft and Gamble, 2007. Chick's liver tissue glycogen content at hatch was determined by a colorimetric method based on the reduction of iodine as described by Dreiling et al. (1991). Relative sodium glucose co-transporter 1 (SGLT-1) gene expression was evaluated using three steps;

1- RNA extraction, cDNA production and real-time polymerase chain reaction: total RNA was isolated using EZ-10 Spin Column Total RNA Mini-Preps Kit (Bio Basic Canada Inc.). RNA samples were quantified using (BioDrop µLite, England) at 260,280 nm. By using 1 µl RNA sample to determine quality and quantity of RNA. The first strand of cDNA was synthesized by using M-MLV Reverse Transcriptase. Reverse transcription reaction was performed using oligo –dT primer (5'-TTTTTTTTTTTTTTT-3'). Each 20 µl reaction mixture contained 2 µl (5x) buffer with MgCl2, 2.5 µl (2.5 mM) dNTPs, 1 µl oligo (dt) primer, 3 µl RNA (2mg/ml) and 0.5-unit reverse transcriptase (BioLabs, England) enzyme. PCR amplification was performed in a thermal cycler programmed at 42C° for 1 hr, 72 C° for 10 min to inactivate the reverse transcriptase, transcriptase, this method

was modified from Carginale et al. (2004), cDNA product was stored at 4 C° until use.

2- Quantitative real time polymerase chain reaction (qPCR): one primer was used for Sodium glucose co-transporter 1 (SGLT-1) gene as target gene. The primers sequences for SGLT-1 gene forward and reverse were used 5'- CAT CTT CCG AGA TGC TGT CA-3' and 5'- AAT TCG GCT GAT CAT TCC AG -3'. β-Actin gene was used as a housekeeping gene for normalizing mRNA levels of the target gene. The primers sequences for β-Actin gene forward, GTG GGC CGC TCT AGG CAC CAA-3' and reverse 5'- CTC TTT GAT GTC ACG CAC GAT TTC-3'. The gene was determined by quantitative RT-PCR; (CFX connect real-time system BIO RAD) with the SYBR Green (green 2-Go qPCR Mastermix, Bio Basic, Canada). All qPCRs reactions were performed in duplicate, the qPCR reaction was carried out in 10 µl. The real time PCR program was as follows: initial denaturation at 95◦C for 10min; 40 cycles of 95◦C for 15 Sec.; annealing at 60◦C for 40 Sec. and extension at 72◦C for 60 sec.

3-Comparative Ct Method Calculation: a widely used method to present relative gene expression is the comparative Threshold cycle (Ct) method also referred to as the $2^{\wedge (-\Delta\Delta Ct)}$ method (Ginzinger, 2002).

Statistical Analysis: Data from all response variables were analyzed using the GLM procedure of Statistical Package for Social Sciences (SPSS®) software program version 20, (SPSS, 2011). Data was analyzed according to two-way ANOVA of SPSS. In ovo injection solutions were represented by 9 treatments.

The statistical model used was as follows:

$$Y_{ijk} = \mu + A_i + R_j + (AR)_{ij} + e_{ijk}$$

Where:

Y_{ijk}= Observed value of the dependent variable.

µ= Overall mean.

A_i= Effect of the in ovo injection of Arg.

R_j= Effect of the in ovo injection of RJ.

AR_{ij}= Effect of the interaction between Arg and RJ

e_{ijk}= experimental random error.

Means differences at $P \leq 0.05$ were tested by Duncan's New Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The results showed highly significant differences among treatments, also the in ovo injection by 1, 2% L-Arg, 10% RJ and 1% L-Arg + 10% RJ has higher hatchability percentage above control (94.79, 95.83, 96.87 and 94.79 %, respectively). The poorest hatchability percentage (66.67%) obtained with 2% L-Arg +20% RJ mixture. These results indicated that 1, 2% L-Arg, 10% RJ or by both lower levels of studied nutrients enhanced hatchability percentages of Hubbard eggs.

The present results confirm the previous findings showed that the administration / in ovo injection of different amino acids, to make good nutritional status for the embryos, improve hatchability percentage of broiler breeder eggs (Al-Shamery and Al-Shuhaib, 2015; Edwards et al., 2016). Al-Asadi (2013) reported that in ovo administration of Arg and lysine in broilers eggs at 18d of incubation increased hatchability percentages. However, some studies showed that these treatments did not affect or decrease hatchability (Eslami et al., 2014; Salmanzadeh et al., 2016).

On the other hand, the in ovo injection using RJ was found to be inconsistent in their results in literature, Moghaddam et al. (2013) concluded that in ovo

administration of RJ or RJ with antibiotics might be an effective method to increase chicks' body weight without deleterious effect on hatchability. Whereas Moghaddam et al. (2014) found that hatchability was decreased. The in ovo injection of other honey products such as pollen extract found to increase the hatchability of broiler eggs (Coskun et al., 2014); bee bread found to improve hatchability of local Sinai eggs (Rizk and Ibrahim, 2014) and propolis found to has not affect the hatchability of broiler eggs (Bozbay et al., 2016).

The result differences in this field may be due to the strain or quantity, quality (extraction methods), and concentration of amino acid/ RJ injected and /or time and position of injection. Amnion is an efficient site for in ovo injection as found by Zhai et al., (2008) and Keralapurath et al. (2010a, b).

Generally, the present results showed that the in ovo injection of Hubbard breeder eggs by 1% L-Arg, 2% L-Arg or 10% RJ improved the egg hatchability by 6.25, 7.29 and 8.33%, respectively, compared with those of non-injected. It seems that the best mixture from both nutrients to use for in ovo injection for improving hatchability percentage was 1% L-Arg + 10% RJ or 2% L-Arg +10% RJ mixtures, the improvements were 6.25 and 3.13%, respectively, compared with those of non-injected.

Hatchling weight: the results of Table 1 found to has significant differences only among RJ main effect on hatchling weight, whereas 20% RJ has heavier significant chick weight (46.16g) compared with that of control (45.32g). The results showed significant differences among treatments. The chick weight significantly ranged from 44.73g (control) to 46.48g. (20% RJ) and 46.37g (2% L-Arg + 20% RJ), respectively. The other treatments had insignificant differences among them in that respect. These later results indicate the important role of in ovo injection with 20% RJ to obtain higher chick weight at hatch.

The present results confirmed the previous findings showed that the administration / in ovo injection of different amino acids improve chick hatch weight (Al-Zuhairy et al., 2013; Shafey et al., 2014; Salmanzadeh et al., 2016; Azhar et al., 2016). Nayak et al. (2016) found that the in ovo injection of Arg has highly significant effect on hatch weight of Cobb chicks.

Chicks weight at hatch improved due to in ovo injection with RJ as showed by Aljumaili (2012) and Moghaddam et al. (2014). The in ovo injection of other honey products such as pollen extract tended to reduce the hatching broiler chicks' weight (Coskun et al., 2014); and Bozbay et al. (2016) found that propolis has not affected hatchling weight. Similarly, to Moghaddam et al. (2014) reported that the positive results obtained may be related to the increased amino acids content of yolk or the possibility that amino acids administration heightened the amino acids utilization of the embryo.

These results indicate that the highest significant superiority in chicks body weight was for 20% RJ in ovo injection treatment.

Relative liver and intestine weights: the results of Table **1** showed that there was no significant difference among studied main factors and treatments for relative liver weight. The relative intestine weight values showed significant differences among studied treatments, while among the two main effects found to be insignificant. Chicks received 2% L-Arg in ovo injection recorded the highest

intestine weight percentage (5.52%), while birds received 2% L-Arg + 10% RJ mixture recorded the lowest value (3.90%) in that respect.

In general, Uni et al. (2003) reported that the weight of the intestine, as a proportion of embryonic weight, increases from approximately 1% at 17 days of incubation to 3.5% at hatch. The present results are in line with several studies showed that in ovo nutrients injection, promote early gut development and improve immune status (Ohta et al., 2001; Tako et al, 2005; Gaafar et al., 2013). Moreover, the in ovo injection of amino acids reduces the danger of some diseases through blocking the deficiency in the concentration of these amino acids in hatching eggs in such away it leads into acceleration of immune response (Gaafar et al., 2013). Moghaddam et al. (2014) concluded that RJ in ovo injection of broiler eggs may be an effective method to increase the hatch chick's body weight and chicks' internal organ weights.

Previously, reported that the increased development of the intestines expected results from the known impacts of Arg on growth-regulatory pathways (Chevally et al., 1998). Also, Arg significantly increased broiler gut weight and length, increased jejunal villi number, increased the weight of the liver, gizzard and bursa gland of the broiler chicks, as found by Edwards et al. (2016).

Generally, the present results showed that the in ovo injection of Hubbard breeder eggs by 1, 2% L-Arg, 10% or 20% RJ have higher relative intestine weight by 0.3, 1.34, 0.64 and 0.16%, respectively over those of non-injected group. However, in interesting observation, the mixture 2% L-Arg +10% RJ had the only reduction in the relative intestine weight (-0.28%) among the other mixtures, compared with the results of control value.

Villi length, width and surface area: the results of Table 2 showed the effect of in ovo injection of nutrients studied on jejunum villi length, width and surface area at day of hatch, indicated a highly significant differences only for villi width and surface area among L-Arg main factor groups. In both traits, the 1% L-Arg group has the highest values in that respect compared with non-injected and 2% L-Arg groups.

Also, significant difference was found among treatments for villi length, width and surface area at day of hatch. The three studied traits at hatch observed the highest means for those injected by 1% L-Arg+ 20% RJ mixture (280.97, 75.64 μm and 10625.13 μm^2 for the length, width and surface area of villi, respectively) compared with other treatments. The significant shorter villi length values were for the all other treatments their values were statistically equal and ranged from 220.81 μm (20% RJ) to 249.90 μm (non-injected). The villi width of L-Arg injection groups (1 and 2%) showed insignificant differences between them, whereas they have higher significant values (73.09 and 70.42 μm, respectively) over the control group value (65.24 μm). The RJ injection groups (10 and20%) observed insignificant differences between them for villi width (67.96 and 69.20 μm, respectively).

Within the four mixtures nutrients injected treatments, the 1% L-Arg+10% RJ and 1% L-Arg + 20% RJ treatments had insignificant higher villi width values (73.49 and75.64 μm, respectively) compared with 1%L-Arg injected group (73.09 μm); however, these values significantly surpassed the villi width values of RJ injected groups. The 2% L-

Arg +20% RJ treatment has lowest villi width value (66.74 μm) among injected treatments, while it insignificant higher than that for non-injected group (65.24 μm).

Figure 1 and 2 showed the histological changes between non-injected group and 1% L-Arg + 20% RJ (the better injected group in absorption surface area) groups, the comparison between figures observed positive effect on absorption surface area at day of hatch for the injected group.

The present intestine change results are in line with the findings of Smirnov et al. (2006) and Bohorquez et al. (2007), they showed that in ovo feeding has advantages to improve intestinal development and digestive capacity. Salmanzadeh et al. (2016) showed that the in ovo injection of glutamine significantly increased villi height, villi width and crypt depth at hatch of Cobb 500 chicks. In ovo administration of L-Arg broiler eggs significantly increased gut weight and length and increased jejunal villi number (Edwards et al., 2016).

In explanation, increasing L-Arg levels in the embryonic phase would lead to an increase in the number of small intestine cells (Lin et al., 2011). This increase caused an increase in nutrient absorption. While Smirnov et al. (2006) and Ospina-Rojas et al. (2013) explained that the increasing of the surface area of small intestine was the result of the increased mucin gene expression by goblet cells.

Considering the Duncan letters of treatment results (Table 2), showed that the treatment levels whatever L-Arg or RJ has insignificant differences on all the studied three traits. Also, the results showed insignificant differences among both levels of L-Arg or RJ and non-injected group in respect of villi length and surface area. However, both of L-Arg levels and 20% RJ were having higher significant values over control value in respect of Villi width. In regard to the four mixtures nutrient injected groups, 1% L-Arg+20% RJ group showed significant better villi length, width and surface area among the four groups and also compared to non-injected group (by 12.43%, 15.94 and 30.32 %, respectively).

Blood glucose concentration: the results of Table 3 showed clearly that there are significant differences among treatment groups on plasma glucose. However, the non-injected group value (233.50 mg/100 ml) has equal statistical values with value of 1% L-Arg +20% RJ and 2% L-Arg + 10RJ treatments (231.00 and 227.50 mg/100 ml, respectively).

Chicken embryo prefers to use glucose rather than fatty acids for energy production as oxygen availability is limited before hatching, glucose oxidation provides more energy than lipid catabolism (Elwyn and Bursztein, 1993).The late-term embryo and neonatal chicken depends on gluconeogenesis from amino acids , resulting in the depletion of muscle protein reserves and the reduction in early growth (Vieira and Moran, 1999). Thus, energy required for hatching activities comes from glucose provided from glycogen in liver, yolk sac membrane, and muscle, resulting in an increase in plasma glucose between pipping and hatch (Yadgary and Uni, 2012).

Salmanzadeh et al. (2012) showed that the in ovo injection of glucose and glucose + magnesium in Cobb 500 breeder eggs caused a significant increase in blood glucose as compared negative and positive control groups at the d1 of age. On the other hand, using Ross 308 breeder eggs van de Ven et al. (2013) showed that glucose was higher in midterm and late

than in early chicks ($P \leq 0.001$), however at the the end of incubation (d21.5 for all 3 hatch groups), glucose concentration mg/dI not affected (ranged 201.67 to 214.18 mg/dI) for the three categories. Qaid et al. (2016) found that glucose level of Cobb500 chicks at hatch with different density was 232 mg/ dI.

Liver glycogen content: the results of Table **3** showed the effect of in ovo injection of studied nutrients on liver glycogen content at day of hatch, indicated significant differences only among RJ main factor and treatment groups. The results showed in ovo RJ with 20% level has significant higher liver glycogen content at day of hatch (0.26 mg/g) compared with that of control group (0.16 mg/g). However, liver glycogen content increased with the increase of L-Arg or RJ level. The results considering the in ovo injection of mixtures showed that their liver glycogen content values were higher than control group value (0.134 mg/g), with significant differences only with 2% L-Arg +10 RJ mixture (0.268 mg/g) and 2% L-Arg +20% RJ mixture (0.277 mg/g) values.

The present results confirm most of previous findings with different in ovo injection studies, that the in ovo injection with different nutrients increase chicks' liver glycogen reserve at hatch (e.g. Shafey et al.,2012).

In explanation, Liver glycogen is the most important energy reserve to sustain embryos during the piping and hatching stages in broiler and turkey (Uni et al., 2005). The pre- hatch period is characterized by oral consumption of the amnion by the embryo, accumulation of glycogen reserves in muscle and liver tissues and glycogenolysis, initiation of pulmonary respiration, abdominal internalization of remaining yolk (Moran, 2007). The glycogen reserves are withdrawn as embryos go through the hatching process, insufficient glycogen and albumen forces the embryo to mobilize more muscle protein for gluconeogenesis thereby reducing early growth and development.

Generally, these results indicate that the in ovo injection of L-Arg and RJ was effective in increasing the liver glycogen content values of Hubbard chicks at hatch, and this trend increase with level of each nutrient and mixing between them. Also, the higher glycogen content groups found to had higher chick weight at hatch (Table 1)

Relative SGLT-1 gene expression at hatch: the results of Table **3** showed the effect of in ovo injection of studied nutrients on SGLT-1 gene expression compared with non-injected treatment of Hubbard broiler chicks at day of hatch, indicated significant differences among all studied treatments. The chicks of 2%L-Arg, 10%RJ,1%L-Arg+10%RJ and 2%L-Arg +10%RJ treatments had significant increase of relative SGLT1 gene expression compared to non-injected group, and the relative SGLT1 gene expressions at hatch were 18.53, 11.79, 24.51, 12.15 and 1.01%, respectively.

SGLT1 gene is known to play an important role in gut apical glucose uptake (Hume et al., 2005). The later relation between SGLT1 gene and gut apical glucose uptake with human and different species of animals, was not showed in the present study according to the relative liver weight, liver glycogen content, blood glucose concentration, relative SGLT1 gene expression results. In contrast, despite lower relative SGLT1 gene expression of 20% RJ (5.28%) and 2% L-Arg + 20% RJ (4.48%) treatments, they had higher liver glycogen content (0.258

and 0.277 mg/g, respectively). These results proposed that SGLT1 gene expression (glucose transporter) in broiler chicken intestine is regulated in a different manner from that in mammals.

The present results are in line with previous findings of Uni et al. (2003), and Kermanshahi et al. (2015), who showed that the in ovo injection of different amino acids increase the expression of different gene transporters.

In explanation, Yalcin et al. (2015) showed greater PepT1 expression than SGLT1 and they suggested that probably related to the importance of proteins during development and may be necessary to maximize amino acid assimilation when the feed become available as reported previously by Mott et al. (2008). It was described that genes are important for functional developments should have the highest expression levels at early life (Schokker et al., 2009). In addition, injection of fertilized eggs with nutrients play an important role in replacing any deficiency in the synthesis of food materials involved in the composition of the egg which may occur as a result of the possible maternal malnutrition (Selim et al., 2012).

In ovo injection improvements: In general, the estimated improvement results of Table 4 showed that the in ovo injection with 10% RJ and 2% Arg has good improvement results for good hatchability percentage with Hubbard breeder eggs, which may recommend to use. However, the in ovo injection with1% Arg+20 % RJ mixture has improvement impact on intestine weight and villi measurements (gut development).

Table (1): Effect of in ovo injection of L-Arg and RJ or their mixtures in fertile eggs of Hubbard breeder on hatchability, chick weight, intestine weight and liver % at hatch (Means ± SE).

Effects		Hatchability (%)		Chick weight at hatch (g)	Liver (%)		Intestine (%)	
		Abs.	Trans. (SE)		Abs.	Trans. (SE)	Abs.	Trans. (SE)
Factors main effect								
L-Arg %	0	90.62	2.04	45.56±0.29	2.97	0.03	4.45	0.04
	1	89.58	2.83	45.72±0.17	2.81	0.03	4.75	0.03
	2	84.72	3.53	45.84±0.24	2.64	0.04	4.70	0.06
Sig.		NS		NS	NS		NS	
RJ %	0	93.06^{α}	2.11	45.32^{β} ±0.20	3.08	0.03	4.72	0.06
	10	94.44^{α}	1.95	$45.64^{\alpha\beta}$ ±0.20	2.71	0.03	4.47	0.04
	20	77.43^{β}	1.85	46.16^{α} ±0.24	2.63	0.04	4.69	0.03
Sig.		****		*	NS		NS	
L-Arg * RJ treatments effect								
0	0	88.54^{bcd}	1.76	44.73^{b} ±0.39	3.74	0.05	4.18^{cd}	0.08
1	0	94.79^{a}	3.96	45.78^{ab} ±0.12	2.78	0.02	4.48^{bcd}	0.06
2	0	95.83^{a}	3.56	45.46^{ab} ±0.26	2.71	0.09	5.52^{a}	0.08
0	10	96.87^{a}	2.94	45.47^{ab} ±0.32	2.61	0.07	4.82^{abc}	0.07
0	20	86.46^{cd}	1.74	46.48^{a} ±0.38	2.55	0.05	4.34^{bcd}	0.05
1	10	94.79^{a}	3.96	45.77^{ab} ±0.52	2.82	0.01	4.70^{abcd}	0.06
1	20	79.17^{d}	1.20	45.62^{ab} ±0.13	2.83	0.08	5.07^{ab}	0.02
2	10	91.67^{abc}	2.91	45.69^{ab} ±0.26	2.69	0.04	3.90^{d}	0.06
2	20	66.67^{e}	1.03	46.37^{a} ±0.59	2.51	0.07	4.67^{abcd}	0.07
Sig.		***		*	NS		***	

Abs.= Absolute values Trans.= Transformed values

Means having different letter (small or latin letters) in the same column are significantly different (P≤0.05).

NS= Not Significant. * = P ≤ 0.05 *** = P ≤ 0.001 **** = P ≤ 0.0001

In ovo injection-L-Arg, RJ-Hatchability-liver glycogen and SGLT1 gene expression.

Table (2): Effect of in ovo injection of L-Arg and RJ or their mixtures in fertile eggs of Hubbard breeder on jejunum villi length[1], width[1] and surface[2] area at hatch **(**Means ± SE).

Effects		Villi		
		Length (μm)	**Width (μm)**	**Surface area (μm^2)**
Factors main effect				
L-Arg %	0	238.84±6.10	67.47^{B} ±0.75	8057.42^{B} ±220.84
	1	252.04±9.09	74.07^{A} ±0.89	9337.92^{A} ±364.37
	2	236.98±4.33	69.30^{B} ±0.67	8214.00^{B} ±182.34
Sig.		NS	****	****
RJ %	0	246.58±5.79	69.58±1.03	8573.50±221.86
	10	236.47±3.83	70.73±0.91	8351.67±110.20
	20	244.82±9.86	70.53±1.40	8684.17±486.74
Sig.		NS	NS	NS
L-Arg * RJ treatments effect				
0	0	249.90^{b} ±6.57	65.24^{e} ±0.46	8153.07^{bc} ±238.48
1	0	247.00^{b} ±12.29	73.09^{ab} ±0.88	9012.18^{b} ±362.46
2	0	242.85^{b} ±12.90	70.42^{bcd} ±0.22	8555.24^{bc} ±479.76
0	10	245.82^{b} ±9.01	67.96^{cde} ±0.16	8351.47^{bc} ±289.98
0	20	220.81^{b} ±10.95	69.20^{cd} ±1.81	7667.81^{c} ±559.63
1	10	228.15^{b} ±4.17	73.49^{ab} ±1.97	8376.12^{bc} ±180.88
1	20	280.97^{a} ±16.20	75.64^{a} ±1.67	10625.13^{a} ±632.65
2	10	235.43^{b} ±3.25	70.74^{bc} ±0.41	8327.44^{bc} ±127.73
2	20	232.67^{b} ±3.32	66.74^{de} ±1.22	7759.21^{c} ±85.26
Sig.		**	*	**

Means having different letter (capital or small letters) in the same column are significantly different (P≤0.05).

NS= not significant. * = P ≤ 0.05 ** = P ≤ 0.01 **** = P ≤ 0.000

1 Values are means ± SE of 3 birds with 45 villi measured for each bird.

2 Surface area was calculated as: (1/2*width) *length.

Table (3): Effect of in ovo injection of L-Arg and RJ or their mixtures in fertile eggs of Hubbard breeder on chicks' blood glucose concentration, liver glycogen content and Relative SGLT-1 gene expression at hatch (Means ± SE).

Effects		Blood Glucose (mg/100 ml)	Liver glycogen content (mg/g)	Relative SGLT-1 gene expression
Factors main effect				
L-Arg %	0	210.42±5.91	0.200±0.031	6.03^{B} ±1.54
	1	215.17±4.47	0.190±0.023	12.46^{A} ±3.40
	2	213.58±5.02	0.250±0.020	11.72^{A} ±2.61
Sig.		NS	NS	*
RJ %	0	213.08±5.34	0.160^{β} ±0.021	9.67^{β} ±3.27
	10	208.92±5.55	$0.211^{\alpha\beta}$ ±0.030	16.15^{α} ±2.42
	20	217.17±4.33	0.261^{α} ±0.021	4.38^{β} ±0.34
Sig.		NS	NS	***
L-Arg * RJ treatments effect				
0	0	233.50^{a} ±6.56	0.134^{c} ±0.039	1.01^{d} ±0.07
1	0	206.00^{cd} ±5.12	0.149^{bc} ±0.049	9.47^{bcd} ±5.04
2	0	199.75^{cd} ±5.39	0.200^{abc} ±0.030	18.53^{ab} ±6.42
0	10	190.75^{d} ±4.59	0.164^{abc} ±0.044	11.79^{bc} ±2.56
0	20	207.00^{cd} ±3.16	0.285^{a} ±0.026	5.28^{cd} ±0.09
1	10	208.50^{cd} ±2.40	0.197^{abc} ±0.016	24.51^{a} ±4.71
1	20	231.00^{ab} ±7.84	0.220^{abc} ±0.052	3.39^{cd} ±0.69
2	10	227.50^{ab} ±9.32	0.268^{ab} ±0.051	12.15^{bc} ±0.70
2	20	213.50^{bc} ±5.69	0.277^{a} ±0.024	4.48^{cd} ±0.44
Sig.		****	****	***

Means having different letter (small or latin letters) in the same column are significantly different (P≤0.05).

NS= Not Significant. * = P ≤ 0.05

Table (4): The improvements as affected by the in ovo nutrients injection (L-arginine (Arg), royal jelly (RJ) or their mixtures) treatments in fertile eggs of Hubbard breeder compared with non-injected.

Treatments Arg*RJ	Hatcha-bility (%)	Chick weight (g)	Intestine Weight (%)	Liver glycogen (mg/g)	Villi Length (µm)	Villi Width (µm)	Surface area (μm^2)	Relative SGLT1 gene expression
0*0	88.54	44.73	4.18	0.134	249.90	65.24	8153.07	1.01
1*0	7.06	2.35	7.18	11.19		12.03	10.54	9.47
2*0	8.23	1.63	32.06	49.25		7.94	4.93	18.53
0*10	9.41	1.65	15.31	22.39		4.17	2.43	11.79
0*20		3.91	3.83	112.69		6.07		5.28
1*10	7.06	2.33	12.44	47.01		12.65	2.74	24.51
1*20		1.99	21.29	64.18	12.43	15.94	30.32	3.39
2*10	3.54	2.15		100.00		8.43	2.14	12.15
2*20		3.67	11.72	106.72		2.30		4.48
Sig.	***	*	****	*	**	*	**	***

* P≤ 0.05 ** P≤ 0.01 *** P≤ 0.001 **** P≤ 0.0001

The empty box: is significant or insignificant with control but this number smaller than it

The normal number: is insignificant with control but this number bigger than it

The underlined bold number: is significant with the control and this number is bigger than it

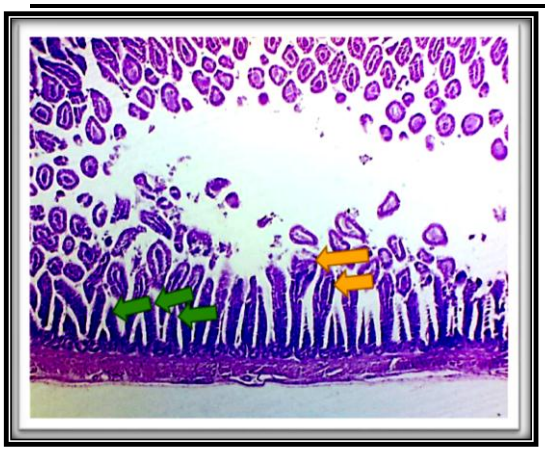

Figure (2): (1% L-Arg + 20% RJ mixture) The figure shows increase in villi number and jejunum villi width and height (green arrows) which had a positive effect on absorption surface area (10625.13 μm^2) at hatch. (StainH&EX100)

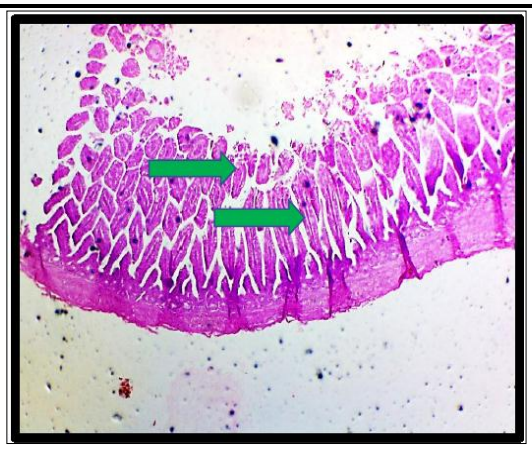

Figure (1): (non-injected group) The figure shows decrease in jejunum villi number, width and height (yellow arrows). Some villi had normal length and some other had less normal (green arrows) of chick's jejunum at hatch, which had effect in lowering the absorption surface area (8153.07 μm^2). (StainH&EX100)

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الملخص العربي

تأثير حقن البيض ببعض العناصر الغذائية علي الاداء الانتاجى وبعض الصفات الفسيولوجية لكتاكيت سلالة الهبرد

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نظرا لأهمية عملية التفريخ والتي تؤثر بشكل مباشرعلي الاداء الانتاجي لدجاج التسمين ، أجريت تجربة لتقييم تأثير حقن بيض التفريخ بمحلول يحتوي علي بعض العناصر الغذائية علي بعض الصفات الانتاجية والفسيولوجية والتعبير الجيني لجين SGLT1 لكتاكيت التسمين (سلالة Hubbard). أجريت الدراسة في وحدة بحوث الدواجن ، كلية الزراعة ، جامعة الإسكندرية ، خلال الفترة من أكتوبر إلى ديسمبر 2015.

أجريت التجربة علي عدد 864 بيضة مخصبة من دجاج سلالة الهبرد Hubbard لدراسة تأثير حقن البيض بثلاث مستويات(0، 1، 2%) من الحامض الاميني أرجنين ، وثلاث مستويات (0،10، 20%) غذاء ملكات النحل أو مخلوط منها وتأثير ذلك علي بعض الصفات الانتاجية والفسيولوجية للدجاج عند الفقس. تم وزن البيض وتقسيمه إلى 9 مجموعات (96 بيضة لكل معاملة وزعت علي اربع مكررات) وعند عند اليوم 18 من عمر الجنين تم الحقن داخل البيض المخصب بالمحاليل المختلفة - حسب كل معاملة- بمعدل 0.5 مل/ بيضة. وكانت اهم النتائج كما يلي:

أظهرت النتائج أن الحقن بالمستويين 1 ، 2 ٪ أرجنين أو 10 ٪ غذاء ملكات النحل قد حسن من نسبة الفقس بالنسب 6.25 ، 7.29 و 8.33 ٪ ، على التوالي مقارنة مع المجموعة الغير محقونة في حين أن المعاملة المحقونة بالمستوى 20 ٪ غذاء ملكات النحل خفضت نسبة الفقس رقميا وليس معنويا مقارنة مع المجموعة الغير محقونة.

كما اظهرت النتائج ان حقن البيض بنسبة 1 ، 2 ٪ أرجنين ، أو 10 ، 20 ٪ غذاء ملكات النحل حسن وزن الدجاج بنسبة (2.34 ، 1.63 ، 1.65 و 3.91 ٪ ، على التوالي) مقارنة مع المجموعة غير المحقونة. كما أظهرت النتائج أن حقن البيضة بنسبة 1 ، 2٪ أرجنين أو 10 ، 20٪ غذاء ملكات النحل كان أفضل بالنسبة للوزن النسبى الأمعاء بنسبة 0.3 ، 1.34 ، 0.64 و 0.16٪ ، على التوالي مقارنة بتلك المجموعة غير المحقونة. ومع ذلك، ادي الخليط 2٪ أرجنين + 10% غذاء ملكات النحل الي الانخفاض الوحيد في الوزن النسبى للأمعاء بالنسبة (-0.28٪) مقارنة مع الخلائط الأخرى ومقارنة بنتائج المجموعة غير المحقونة.

أشارت النتائج إلى أن ان حقن البيض كان فعالا في زيادة محتوى الجليكوجين في الكبد عند الفقس، وهذا الارتفاع في محتوى الجليكوجين استمر مع زيادة زيادة مستوى كلا من أرجنين و غذاء ملكات النحل والخليط بين كل منهما.

ظهرت النتائج أن كل معاملات الحقن أدت الي اختلافات طفيفة في طول وعرض الخملات وكانت مستويات الارجنين المختلفة لها تأثير أفضل علي عرض الخملات بالمقارنة بمستويات غذاء ملكات النحل علي خملات امعاء الطيور عند الفقس ومع ذلك ، كان كل من (1 و 2 ٪) أرجنين أكثر فعالية من كلا (10 و 20 ٪) غذاء ملكات النحل فيما يتعلق بعرض الخملات.

أشارت النتائج إلى أن الحقن بمستوي 2% أرحنين أو مخلوط 1%أرجنين مع 10% غذاء ملكات النحل أدي إلي زيادة التعبير الجيني لجين (SGLT-1) بالمقارنة بالكنترول وباقي المعاملات عند الفقس.

وبشكل عام فإن حقن البيض بنسبة 1٪ أرجنين أو خليط من 1٪ أرجنين +10٪ غذاء ملكات النحل يمكن ان يوصى بأستخدامه مع بيض سلالة (Hubbard) من اجل تحقيق نتائج جيدة لصفات الفقس كما أن الحقن بمخلوط 1% أرجنين+ 20% غذاء ملكات النحل يمكن ان نوصي به لتحقيق افضل تطور للامعاء عند الفقس.