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EFFECT OF *IN OVO* NANO-SELENIUM AND GLUTAMINE ON HATCHABILITY, POST-HATCH PERFORMANCE AND SOME BLOOD BIOCHEMICAL INDICES IN JAPANESE QUAIL

[185]

Sallam[1*] M.G., El-Wardany[2] I., Abdel-Fattah[2] S.A., El-Mallah[1] G.M. and Yassein[1] S.A.

1- Animal Production Dept., Agric. and Biological Research Division, National Research Centre, Giza, Egypt
2- Poultry Production Dept., Fac. of Agric., Ain Shams Univ., P.O. Box 68, Hadayek Shubra 11241, Cairo, Egypt

*Corresponding author: mohamed_sallam@agr.asu.edu.eg

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ABSTRACT

A total number of 500 Japanese quail fertile eggs were used to study the effect of *in ovo* (IO) administration of Nano-Selenium (Nano-Se) and Glutamine (Gln) on hatchability, post hatch performance carcass and some blood biochemical traits. Eggs were divided into five treatments groups and injected just before incubation. The 1st group was not injected (Negative Control;T1), the 2nd one was injected with Bovine Serum Albumin (BSA) (Positive control; T2), the 3rd group was injected with Nano-Se at 2.5 ppb / egg (T3), while, the 4th (T4) and 5th (T5) groups were injected with glutamine at 20 and 10 ppm / egg, respectively. The hatched chicks were distributed according to their treatments and reared up to the 6th week of age. Parameters such as the hatchability, hatchlings weight, productive performance, carcass traits and some blood biochemical indices were estimated. The IO-Gln at 20 ppm (T4) significantly improved hatchability, increased the productive performance of post-hatch quails (carcass weight, gizzard and breast muscles percentages) and enhanced the feed conversion ratio (FCR) compared to other groups. Furthermore, IO with Nano-Se (T3) significantly increased plasma albumin and albumin / globulin (A/G) ratio, and decreased cholesterol and triglycerides levels, while high density lipoprotein (HDL) were significantly increased and low density lipoprotein (LDL) were significantly decreased by IO-Gln (T4 and T5). It could be concluded that using the *in ovo* administration of Nano-Se and Gln concentration for Japanese quail breeder eggs, had no negative effect on hatchability and could improve post-hatched performance carcass traits, particularly using Gln at a dosage of 20 ppm / egg.

Keywords: *In Ovo*, Nano-Selenium, Glutamine, Hatchability, Performance, Quail

INTRODUCTION

In ovo (IO) considers the method of providing exogenous substances for the avian embryos **(Uni and Ferket, 2003)**, that improve the chicks performance from injected eggs **(Salmanzadeh, 2011; Salmanzadeh et al 2012 and Dong et al 2013).** The methods of *in* ovo materials ensure an acceptable nutrient content in egg with injected nutrients that increase hatching weight and supply embryos with extra nutrients and energy **(Uni et al 2005 and Foye et al 2006a). Uni and Ferket (2003)** considers the *in ovo* injection technology is a safety method to conduct the external materials to embryonic development. Also, during the incubation, the injected materials are swallowed, digested and absorbed by the embryo, respectively before piping **(Uni et al 2005)**.

Selenium (Se) is the essential micro-nutrient plays an important role in a number of biological processes, activates glutathione peroxidise and seleno-enzymes which can help in protecting from the free radicals, which destroy body cells and reducing the immunity. The introduction of IO-Se to the incubating embryo was found to be a suitable alternative. Since, Se deficiency in poultry diets caused several pathological conditions that can

harm growth and development. *In ovo* nanoparticles acting as active agents and carriers of nutrient that helps of nano-nutrition to support embryos with bioactive substances **(Sawosz et al 2012).**

Glutamine (Gln), one of the amino acids that involved in the protein and carbohydrate metabolism and are a distinct supplier of the amino group for endogenous amino acids synthesis. Furthermore, Gln is the major motive power for gastrointestinal tract development **(Andrew and Griffiths, 2002)**, It is also reduce high ammonia levels from protein catabolism, necessary for uric acid synthesis and a source of arginine **(Bertolo and Burrin, 2008).** Gln is the main source for rapidly proliferating cells specially activated lymphocytes and intestinal enterocytes **(Calder and Yaqoob, 1999)** besides a small intestine barrier of the mucus **(Le Bacquer et al 2003)**. Also, amino acids are essential for glycogen synthesis that limits for protein synthesis owing to limited carbohydrate storage in the eggs **(Sunny et al 2007).**

Thus, the present study illustrates the response of Japanese quail breeder eggs subjected to *in ovo* administration with nano-selenium or glutamine on hatchability, post-hatch performance, carcass yield, digestive organs (including: small intestine, liver, gizzard), breast muscles and some blood plasma constituents.

MATERIALS AND METHODS

The present study was performed at the Quail Production Unit, Agricultural Experiments and Research Station, Fac. of Agric., Ain Shams Univ. Farm at Shalaqan, Qalyubia, Egypt, during the period from May to July 2017.

Experimental Design

A total number of 500 fertilized fresh eggs of 12 weeks old Japanese quail breeders flock were used in this study. A day before setting in the incubator, eggs were numbered, weighed individually and randomly divided into five experimental groups (100 eggs / group) and subjected to injection in air cell just before incubation as follows: The 1st group (T1) was used negative control and not injected; the 2nd was considered as (T2) positive control injected with bovine serum albumin (BSA) that was used to dissolve Nano-Se. The 3rd (T3), 4th (T4) and 5th (T5) groups were received Nano-Selenium (2.5 ppb / egg), Glutamine (20 ppm/egg), Glutamine (10 ppm / egg), respectively.

Experimental procedures

Nano form of selenium was prepared by adopting the procedure of **Razi et al (2011),** while the concentration of glutamine in the *in ovo* injection solution was formulated to the amino acid composition of albumin by **Belitz et al (2009).** The open holes of injected eggs were covered by non-toxic glue according to **Bhanja (2004)**.

Measurements and observations

Hatchability percentage

Hatchability percentage was calculated based on the number of hatched chicks as a percentage of the *in ovo* treated eggs at hatch. Also, chicks weight were recorded.

Birds and Data collection

The hatched chicks of all treatments were individually weighed, a total number of 45 birds for each treatment were randomly taken and assigned to three replicates (15 / replicate). Chicks of all treatment groups were housed in galvanized cages up to the age of six weeks. Water and feed were let for *ad libitum* consumption. Feed was formulated to meet the recommendations of **NRC (1994)**. Generally, all chicks were kept under similar managerial and hygienic conditions.

Measurements: hatchability percentage was calculated at the end of incubation period based on the number of fertile eggs. Live body weight (LBW) and feed intake (FI) were recorded for bi-weekly interval throughout the growth period, however the average final body weights were presented weight gains (WG) were calculated. At the end of the experiment period (6 weeks), 35 quails (7 quails / treatment) were randomly taken, slaughtered, defeathered, opened and the weights of eviscerated carcases were recorded. Breast muscles and the internal organs (liver, gizzard and small intestine) were removed and separately weighed and proportionated to pre-slaughtering body weight. Blood samples were collected at slaughtering time in heparinized tubes. Plasma were harvested after centrifugation (4000 rpm for 15 min.), then stored at -20ºC until the analyses of some blood constituents were done.

Plasma Total Protein was determined according to **Tietz (1994)**. Albumin was determined according to **Tietz (1990)**. Globulin values were calculated by subtracting albumin values from total

protein values for each replicate within each treatment. Albumin / Globulin ratio (A / G ratio) values were calculated by dividing albumin values on globulin values for each replicate within each treatment.

Cholesterol concentration was determined according to **NCEP (1988)**. Triglycerides were determined according to **Young (1975)**. HDL Cholesterol was determined according to **Lopes-Virella (1977)**. LDL Cholesterol values were estimated by the following formula according to **Friedewald (1972)**:

$$\textit{LDL Cholesterol} = \textit{Total Cholesterol} - \frac{\textit{Triglycerides}}{5} - \textit{HDL Cholesterol}$$

Statistical analysis

Data were statistically analyzed using Analysis of Variance procedure using the General Linear Model (GLM) of **SAS (2002)** using the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = The observed value of a given dependent variable.

μ = Overall mean.

T_i = The effect of treatment

e_{ij} = The experimental error

The differences among means were tested using Duncan's multiple range test **(Duncan, 1955)**.

RESULTS AND DISCUSSION

The results obtained from IO (Nano-Se and Gln) are shown in **Fig. (1)**. It is observed that the IO-Gln at 20ppm/egg (T4) had the highest hatchability percentage while, the lowest percentage was recorded by Nano-Se treatment (T3) compared with the other experimental groups. The results of this study confirm the results of **Uni *et al.* (2005)** who proved that IO-Gln indicates a positive effect during the late period of embryogenesis on increasing the hatchability percentage. Whereas, no effect was noted on hatchability when used IO- Gln at the 18th day of incubation as reported by **Pedroso et al (2006b)**. In this connection, the newly hatched chicks were not affected by using 0.5ml of 10% IO-Gln solution **(Dos Santos et al 2010)**. However, improving energy status of embryos and muscles protein from mobilization as a result for IO-Gln helps to improve hatchability and growth as cleared by **Tako et al (2004a)**; **Tong and Barbut (2004)**; **Uni et al (2005)**; **Foye et al (2006b)** and **Shafey et al (2010)**. The hatchability results of injecting Nano-Se in our study was nearly coincided with those of **Patric et al (2016)** who proved that IO-Nano-Se at 5 ppm level did not significantly influence the hatchability %. In general, as reported by **Uni and Ferket (2004)** showed that the reaction of IO rely on heredity, hatching eggs, conditions of incubation and the eggs size.

Results presented in **Table (1)** shows the response of IO Nano-Se and Gln on performance of Japanese quail chicks. A significant increase existed in final body weight (BW) and body weight gain (BWG) in the treatments of positive control (T2) and Gln (T4 and T5) than the negative control (T1) and Nano-Se (T3).

In this respect, several previous studies showed that, to increase the growth performance of newly hatched-chicks, it is important to stimulate the development of chick embryo **(Tako et al 2004b; Uni et al 2005 and Smirnov et al 2006)**. The feed intake (FI) of IO-Gln (T4) was significantly decreased compared to the other treatments. Results of FCR showed that, it was improved ($p<0.0001$) by IO of Gln at 20 ppm, followed by Gln at 10 ppm, and the Nano-Se injection revealed intermediate FCR comparable to other groups.

It had been reported by **Bartell and Batal (2007) and Salmanzadeh and Shahryar (2013b)** that IO-Gln enhanced FCR and WG of broilers and Japanese quails due to the improvement of intestine. In this connection, **Mehta et al (2016)** indicated that chickens from IO-Gln showed a significantly greater BW and better WG of newly hatched chicks than those of the non-injected eggs. Whereas the IO-Nano-Se had no significant effect on broiler chicks performance **(Abd-El-Fattah et al 2018)**.

The effect of IO injection on carcass traits is shown in **Table (2)**. It was observed that IO injection of Japanese quail eggs with Gln with either 20 or 10 ppm, just before incubation period had increased the percentages of eviscerated carcass significantly and breast muscles of chicks at 6 weeks of age when compared with the negative control group. The IO-Nano-Se (T3) realized nearly similar previous trend obtained with Gln, also it increased significantly small intestine percentage. Similarly, IO-Gln had led to increase the small intestine percentage, however, the absence of significance compared to the negative control. It is clear that liver percentages were slightly elevated with the injection of tested materials comparable to the non-injected group.

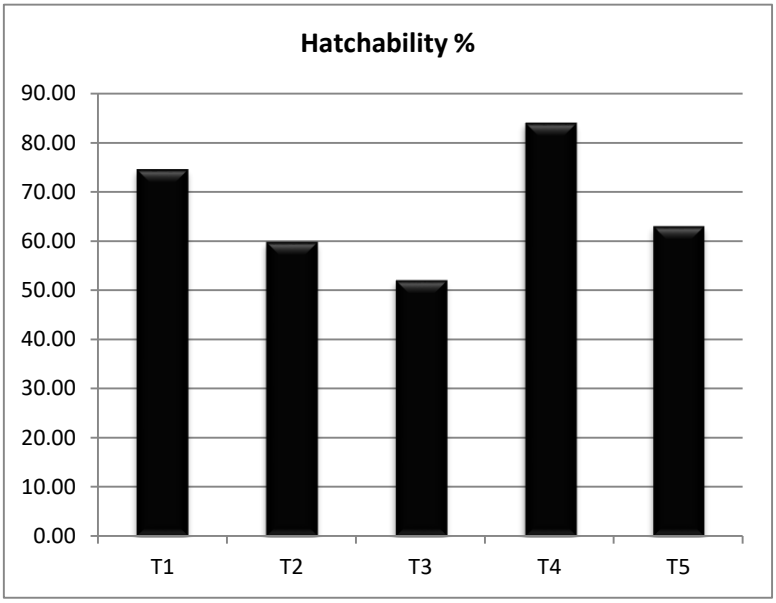

Fig. 1. Effect of *In Ovo* injection of Nano-Selenium and Glutamine on hatchability percentage

Table 1. Effect of *In Ovo* injection of Nano-Selenium and Glutamine on Performance of post-hatch chicks

Trait	Treatment					P. Value
	T1	T2	T3	T4	T5	
Live Body Weight (gm)						
Initial Weight (Day Old)	8.73±0.12	8.78±0.08	8.81±0.18	8.58±0.05	8.80±0.08	0.5827
Final Weight (6 Weeks)	196.19^{b}±1.95	224.17^{a}±3.02	201.71^{b}±2.89	231.23^{a}±3.53	233.13^{a}±4.12	<.0001
Weight Gain (gm)						
0-6 Weeks	187.46^{b}±1.92	215.39^{a}±2.98	192.90^{b}±2.93	222.65^{a}±3.46	224.33^{a}±4.08	<.0001
Feed Intake (gm)						
0-6 Weeks	737.33^{b}±4.33	917.33^{a}±8.74	721.33^{b}±10.41	638.33^{c}±10.14	731.67^{b}±1.76	<.0001
Feed Conversion						
0-6 Weeks	3.94^{a}±0.06	4.12^{a}±0.02	3.75^{b}±0.001	2.85^{d}±0.12	3.31^{c}±0.07	<.0001

a,b,c,....: In each row means having different letters are significantly different ($p<0.05$).
(T1) Negative Control (no injection); (T2) Positive Control (solvent, BSA); (T3) Nano-Selenium (2.5 ppb/egg); (T4) Glutamine (20 ppm/egg); (T5) Glutamine (10 ppm/egg).

Table 2. Effect of *In Ovo* injection of Nano-Selenium and Glutamine on Carcass traits

Trait	Treatment					P. Value
	T1	T2	T3	T4	T5	
Carcass%	65.93^{b}±0.96	70.24^{a}±1.56	70.01^{a}±0.58	72.95^{a}±1.77	73.74^{a}±1.12	0.0020
Gizzard%	1.76^{b}±0.08	1.77^{b}±0.09	1.68 ^{b}±0.03	1.91^{b}±0.06	2.28^{a}±0.16	0.0105
Liver%	2.11^{c}±0.12	2.79^{a}±0.13	2.65^{ab}±0.36	2.42^{b}±0.12	2.59^{ab}±0.17	0.0543
Empty Small Intestine%	4.16^{b}±0.28	6.03^{a}±0.16	5.65^{a}±0.33	4.61^{b}±0.27	4.71^{b}±0.09	0.0002
Breast Muscles%	18.76^{b}±0.25	19.72^{b}±1.14	19.99^{b}±0.62	23.52^{a}±1.01	23.36^{a}±1.5	0.0080

a,b,c....: In each row means having different letters are significantly different (p<0.05).
(T1) Negative Control (no injection); (T2) Positive Control (solvent, BSA); (T3) Nano-Selenium (2.5 ppb/egg); (T4) Glutamine (20 ppm/egg); (T5) Glutamine (10 ppm/egg).

Table 3. Effect of *In Ovo* injection of Nano-Selenium and Glutamine on Blood traits

Trait	**Treatment**					**P. Value**
	T1	**T2**	**T3**	**T4**	**T5**	
Total Proteins (g/dl)	3.61±0.08	3.84±0.18	3.89±0.24	3.44±0.17	3.69±0.12	0.3606
Albumin (g/dl)	1.36^{bc}±0.04	1.54^{ab}±0.06	1.64^{a}±0.10	1.27^{c}±0.07	1.43^{bc}±0.04	0.0039
Globulin (g/dl)	2.26±0.08	2.29±0.14	2.23±0.13	2.17±0.11	2.26±0.08	0.9583
A/G ratio	0.602^{c}±0.032	0.672^{ab}±0.022	0.735^{a}±0.019	0.585^{c}±0.013	0.633^{bc}±0.010	<.0001
Cholesterol (mg/dl)	133.39^{ab}±6.08	142.21^{a}±4.37	125.13^{b}±4.23	135.04^{ab}±2.38	127.80^{ab}±5.59	0.03516
Triglycerides (mg/dl)	64.83^{bc}±3.61	86.91^{a}±1.39	60.75^{c}±3.86	74.81^{ab}±5.06	81.37^{a}±5.01	0.0021
HDL (mg/dl)	75.64^{b}±5.14	72.56^{b}±4.44	64.53^{b}±3.20	114.12^{a}±1.22	103.61^{a}±4.5	<.0001
LDL (mg/dl)	59.71^{a}±3.48	47.63^{b}±3.96	43.04^{bc}±3.29	35.36^{cd}±3.68	29.5^{d}±1.85	0.0004

a,b,c,....: In each row means having different letters are significantly different (p<0.05).
(T1) Negative Control (no injection); (T2) Positive Control (solvent, BSA); (T3) Nano-Selenium (2.5 ppb/egg); (T4) Glutamine (20 ppm/egg); (T5) Glutamine (10 ppm/egg).

Our results on breast muscle are similar to those findings of **Chen et al (2013)** who found that IO-Gln resulted in a significant increase in the breast muscles size during the incubation period compared to the control. Also, **Yi et al (2005)** and **Fischer da Silva et al (2007)** found that Gln can stimulate the small intestine development in poults. In this respect, **Le-Bacquer et al (2003)** reported that the Gln supplement is very important to protein structure of mucus and the formation of small intestine barrier.

On the other hand, the significant increased in carcass % as a result of IO-nano-Se (2.5 ppb / egg) was similar to those of **Patric et al (2016**) who showed that the IO-Nano-Se of broiler eggs cleared significantly highest carcass % and had no significant effect on breast muscle % as compared to control.

Results in **Table (3)** showed insignificant differences among all experimental groups concerning plasma total protein and globulin. While, albumin concentration was elevated (p≤0.004) in quail chicks whose eggs were IO injected with Nano-Se at 2.5 ppb / egg, when compared with chicks of all remaining treatment groups. Similar trend was obtained for A/G ratio data. These results may be due to increasing the BWG by increasing the IO-Gln (T4 and T5).

Similar reports were observed by **El-Said (2015)** who found no significant effect of globulin with group of Nano-Se 20 ppb and **Selim et al (2015)** who showed that no significant difference at 0.15 and 0.30 ppm in diet or drinking water on plasma total protein and globulin. Whereas, supplementing Nano-Se increased plasma concentration of total protein, albumin and significant difference in globulin **(Abd El-Fatah et al 2018)**. On the other hand, **Kanagaraju and Rathnapraba (2017)** reported similar results that IO-Gln had no significant differences in plasma total protein, albumin and globulin compared with sham control but had significant differences in the same parameters compared with control group.

Results of plasma lipid profiles showed that, chicks derived from eggs that were injected with Nano-Se recorded insignificantly lesser plasma concentration of cholesterol, triglycerides and HDL compared to chicks of non-injected group. But when compared with Gln chicks group, Nano-Se chicks had significantly lower triglycerides and HDL. Injection of Japanese quail breeder eggs at a period of pre-incubation resulted in significant elevation in the plasma concentration of HDL in the blood of produced chicks at 6 week of age comparable to the chicks produced from non-injected eggs. Significant higher concentration of LDL were obtained for negative and positive groups than the groups of Nano-Se and Gln.

This result is in agreement with that reported by **Abd El-Fatah et al (2018) and Saleh (2014)** who cleared that using Nano-Se had no significant differences on plasma cholesterol and triglycerides levels. However, these results disagree with that reported by **El-Said (2015)** who found a significant increase in HDL when used Nano-Se at 40 ppb, but no effect was obtained with group received Nano-Se at 20 ppb.

CONCLUSION

In conclusion, it could be concluded that using *in ovo* administration of Gln and Nano-Se concentration for Japanese quail breeder eggs, had no negative effect on hatchability and could improve post-hatched performance carcass traits, particularly using Gln at a dosage of 20 ppm / egg.

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تأثير الحقن بالنانو سيلينيوم والجلوتامين على الفقس، الأداء الأنتاجي بعد الفقس وبعض مؤشرات الدم البيوكيميائية في السمان الياباني

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محمد جمال سلام[1*] – إبراهيم الورداني[2] – سيد أحمد عبد الفتاح[2] –
جمال منصور الملاح[1] – سعد الدين أحمد يس[1]

1– قسم الإنتاج الحيواني– شعبة البحوث الزراعية والبيولوجية– المركز القومي للبحوث– الجيزة – مصر
2– قسم إنتاج الدواجن– كلية الزراعة– جامعة عين شمس– ص.ب. 68 – حدائق شبرا 11241– القاهرة– مصر
*Corresponding author: mohamed_sallam@agr.asu.edu.eg

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الموجـــــز

الهدف الرئيسي من هذه الدراسة هو قياس نسبة الفقس والأداء بعد الفقس مثل وزن الجسم ومعدل التحويل الغذائي ومواصفات الذبيحة وبعض القياسات البيوكيميائية بإستخدام بيض أمهات السمان الياباني المخصب وحقنه بالنانوسيلينيوم والجلوتامين.

تم أستخدام 500 بيضة مخصبة، ثم تقسيم البيض إلى خمس مجموعات وحقنه قبل التحضين مباشرة كالأتي: الأولي أستخدمت بدون حقن (المقارنة السلبية)، والثانية حقنت بـ (Bovine Serum Albumin) (المقارنة الأيجابية)، تم حقن المجموعة الثالثة بالنانوسيلينيوم بتركيز 2.5 جزء في المليار لكل بيضة، بينما تم حقن المجموعتين الربعة والخامسة بالجلوتامين بتركيز 20 و 10 جزء في المليون لكل بيضة على التوالي. وتم توزيع الكتاكيت بعد الفقس وفقًا لمجموعتها بتكرارتها حتي عمر 6 أسابيع. ثم تم أخذ بعض القياسات مثل نسبة الفقس، وزن الكتاكيت الفاقسة، الأداء بعد الفقس مثل بعض صفات الذبيحة وبعض المؤشرات البيوكيميائية للدم.

وجد أن حقن البيض بالجلوتامين بنسبة 20 جزء في المليون لكل بيضة قام بتحسين نسبة الفقس، وزيادة وزن الجسم الحي ومعدل زيادة الوزن للسمان بعد الفقس، وزيادة نسبة وزن الذبيحة والقونصة وعضلات الصدر، ومعدل التحويل الغذائي بالنسبة لباقي المجموعات. وعلاوة علي ذلك، الحقن بالنانوسيلينيوم زاد من ألبيومين الدم ونسبة الألبيومين للجلوبيولين وخفض نسب الكوليستيرول والدهون الثلاثية، بينما تم زيادة الـ HDL وإنخفاض الـ LDL عند حقن البيض بمستويات الجلوتامين في المجموعتين الرابعة والخامسة. ونستنتج من ذلك أن الحقن بالنانو سيلينوم وتركيزات الجلوتامين لم يؤثر بالسلب علي الفقس وربما يكون له تأثير جيد علي الأداء الإنتاجي بعد الفقس ومعاملات الذبيحة وخاصة الجلوتامين بنسبة 20 جزء في المليون لكل بيضة.

الكلمـــات الدالـــة: حقــن البــيض، النانوســيلينيوم، الجلوتامين، نسبة الفقس، الأداء الإنتاجي، السمان

تحكيم: ا.د عبدالخالق أبوالفتوح الغمري
ا.د نعمة الله جمال الدين