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EFFECT OF ASCORBIC ACID INJECTION IN PRE-INCUBATED HY-LINE LAYER EGGS ON HATCHABILITY AND SOME BLOOD AND HEMATOLOGICAL PARAMETERS OF HATCHED CHICKS

A.M. Badran[1] ; M. H. Hatab[2] and N. S. Ibrahim[2]

[1]Anim. Prod. Res. Inst., Agric. Res. Center, Ministry of Agric. Dokki, Giza

[2]Biological Applications Dep., Nuclear Res. Center, Atomic Energy Authority, P.O. Box 13759, Egypt

Corresponding author:aamel555@gmail.com

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ABSTRACT: The present work was carried out to study the effect of ascorbic acid (AsA) injection into fertile Hy-line layer eggs on hatchability, mortality and some blood and hematological parameters of hatched chicks. Three hundred Hy-line layer eggs were divided into four equal groups with 75 eggs for each group with three replicate (25 fertile eggs for each replicate). The first group was injected, pre-incubation, in the air cell by 50 µl deionized water and served as a sham control. While the second, third and fourth groups were injected in the air cell with either 2, 4 or 6 % ascorbic acid. dissolved in 50 µl deionized water and served as AsA-treated groups.

Eggs were incubated at 37.5°C and 60 % relative humidity during the first 19th days of incubation. Eggs were turned automatically every 2 hour until the 19th day. At the 10th day of incubation, eggs were examined by light candling to remove infertile eggs . All eggs were transferred to the hatchery at the end of the 19th day of incubation, and placed in hatching trays at 37°C temperature and 70-75 % relative humidity until hatching.

hatchability rate, hatching and hatched chick's body weight unlike mortality were significantly higher in the 6 % AsA groups than all the AsA groups or the control group. Hemoglobin concentration was significantly ($p<0.05$) higher in the the 4 % AsA group and the 6 % AsA group ($p<0.05$) as compared to the 2 % AsA or control groups. However there was no significant difference in the cholesterol concentration between the control group and all the AsA groups. Glucose and triiodothyronin concentrations were significantly ($p<0.05$) higher in the 6 % AsA group than the 2 % AsA or control groups. In conclusion, our study suggests injecting 50 microliters of 6 % ascorbic acid reduced embryonic mortality and improvd hatchability and hatched chicks'body weight.

Key words: In-ovo injection, ascorbic acid, hatchability, embryo mortalities, blood parameters

INTRODUCTION

The subsequent development of avian embryos and hatched chicks are influenced by the yolk nutrient status (Al-Murrani, 1982). Many nutrients have important structural, physiological, and immunological roles in avian embryogenesis and growth performance. In-ovo injection of nutrients may help overcome any constraint of inadequate egg nutrition. During early development, there is rapid oxidative metabolism that leads to the production of large quantities of free radicals in many tissues, making them more susceptible to oxidative damage. Antioxidants are a critical defense against these free radicals. The developing embryo may use antioxidants found in the yolk in order to reduce the impact of free radicals. There are several reports regarding the effect of ascorbic acid (AsA, vitamin C) as an anti-stress agent on productive performance parameters in birds such as growth and reproductive traits, the most important of them are fertility and hatchability. In incubated eggs, chick embryos may be subjected to stress caused by excessive production of metabolic heat during the latter part of egg incubation so, the addition of vitamin C as an anti-stress agent may be beneficial for embryos viability and to protect them from any stress during incubation (Tullett, 1990).

Also, the in-ovo injection of nutrients may be used to improve hatchability and hatchling quality (Ohta et al., 2001). For instance, the injection of vitamins in ovo has been applied to improve hatchability and hatchling body weight (Robel and Christensen, 1991 and Robel, 1993). Nowaczewski et al. (2012) reported that, when eggs are incubated under normal conditions, ascorbic acid has shown, dose-dependent, positive effects on hatchability and hatchlings body weight. Zakaria et al. (1998) reported that vitamin C injected into chicken eggs was demonstrated to have a favourable impact on hatchability results, embryo weight at different incubation days and, consequently, on chick body weight after hatching as well as on the reduction of embryo death during incubation.

In addition, the use of ascorbic acid as a nutritional additive supplement during the fetal stage has shown dose-dependent positive effects of this vitamin on hatchability and body weight at hatching (Ghonim et al., 2009; Mohammed et al., 2011 and Nowaczewski et al., 2012). Ascorbic acid has been demonstrated to improve immunoresponsiveness and increase disease resistance in poultry by optimizing the immune system (Pardue and thaxton, 1986 and Rund, 1989).

There is no data in the literature on the effects of pre-incubation in-ovo injection of vitamin C on incubation and hematological parameters of chicks hatched from eggs incubated under thermoneutral conditions. Therefore, the present study examined the effects of pre-incubation in-ovo injection of ascorbic acid (AsA) on hatchability and some blood and hematological parameters of the hatched chicks.

MATERIALS AND METHODS

The present work was carried out at the poultry research unit, Biological Applications Department, Nuclear Research Center, Egyptian Atomic Energy Authority. The effect of pre-incubation in-ovo injection of ascorbic acid (AsA) on hatchability and some hematological parameters of the hatching chicks was studied. Three hundred Hy-line layer eggs were cleaned with ethanol pre- incubation, then individually weighed (65 ± 2g) and divided into four

equal groups with 75 eggs for each group with three replicate (25 fertile eggs for each replicate). The first group was injected in the air cell by 50 µl deionized water and served as a sham control. While the second, third and fourth groups were injected in the air cell with either 2, 4 or 6 % ascorbic acid (AsA) dissolved in 50 µl deionized water and served as AsA-treated groups.

Eggs were incubated at 37.5°C and 60 % relative humidity during the first 19 days of incubation. Eggs were turned automatically every 2 hour until the 19th day. At The 10th day of incubation, eggs were examined by light candling to remove infertile eggs . All eggs were transferred to the hatchery at the end of the 19th day of incubation, and placed in hatching trays at 37°C temperature and 70-75 % relative humidity until hatching.

Hatchability (number of hatched chicks / number of total hatching eggs) and hatchability of the fertile eggs (number of hatched chicks / number of fertile eggs incubated), hatched chicks'body weight and embryonic mortalities were obtained.

At hatch, blood samples were collected from fifteen chicks per group (five hatchlings per replicate) by decapitation. Blood samples were divided into two tubes, the first with anticoagulants to determine some blood hematological parameters such as hematocrit (HCT) (Harmon, 1936), hemoglobin (HGB) (Harmon, 1936), total red blood cells count (RBC) (Lucas and Jamroz, 1961) and mean corpuscular volume of erythrocytes (MCV). While the second tubs without anticoagulants and stored at normal room temperature for half an hour to allow clotting of the blood. Then the blood was centrifuged to separate the serum. Serum was then frozen and stored at –20°C until assayed.

Serum glucose and cholesterol concentrations were determined colorimetrically using a commercial kit produced by Stanbio Company, USA and measured on computerized spectrophotometer model Milton Roy Spectronic 1201 (1201 Ivyland Road Ivyland, PA 18974, United State). While, triiodothyronin (T_3) concentration was determined using radioimmuno assay (RIA) Kit bought from IZOTOP Company (INSTITUTE OF ISOTOPES Ltd.) (http://www.izotop.hu) and samples were counted on a Pacard Gamma Counter model 540501 RIA SAR.

Statistical analysis

One-way analysis of variance was used to determine the effect of ascorbic acid (AsA) injection in Hy-line layer eggs on hatchability and some blood parameters. Data were statistically analyzed by the General Liner Model Procedure of the SAS software (SAS Institute, 2002). Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) when significant differences at ($P< 0.05$) existed.

The model used was:

$Yi = \mu + Ti + Ej$

Where:

Yi = any value from the overall population.

µ = the overall mean.

Ti = the effect of the ^{i}th treatment (i=1, control& 2, AsA treatment).

Ej = the random error associated with the ^{j}th individual.

RESULTS

Table (1) shows The effect of pre-incubation in-ovo injection of ascorbic acid (AsA) on hatchability, hatchability of fertile eggs, embryonic mortalities percentage and hatched chicks' body weight. The data shows that hatchability and hatchability of fertile eggs were

significantly higher ($p<0.05$) in the AsA groups than the control group and highly significantly increased in the 6 % AsA group than the other AsA groups. While, mortality percentage was significantly decreased in the AsA groups than the control group and significantly lower in the 6 % AsA group than the other AsA groups. Finally, hatched chicks' body weight was was significantly higher in the AsA 6 % group than other AsA groups, while there were no significantly different between the control and the 2 or 4 % AsA groups. The effect of pre-incubation in-ovo injection of ascorbic acid on some hematological parameters is shows in Table (2). Data shows that however hemoglobin concentration (HGB) was not significantly differ between the control group and the 2 % AsA group, its concentration was significantly increased in the the 4 % AA and highly significantly increased in the 6 % AsA. While, hematocrit percentage (HCT %), red blood cell counts (RBC) and mean corpuscular value (MCV) were not significantly different between the control and all the AsA groups. Table (3) shows the effect of pre-incubation in-ovo injection of ascorbic acid on serum cholesterol, glucose concentrations and triiodothyronin level. The data indicated that there was no significant difference in the cholesterol concentration between the control group and all the AA groups. However, glucose and triiodothyronin concentrations were significantly higher in the 6 % AsA group than the 2 % AsA group, there were no significantly differences between the control group and the 2 % AsA group.

DISCUSSION

Hatchability, mortality rates and hatched chicks'body weight:

One of the basic biological functions of the egg shell for the domestic fowl is to allow for adequate movement of water vapor and respiratory gases. It consists of overlaying cuticle which is penetrated by thousands of microscopic pores which are essential for the exchange of respiratory gases during incubation (Tullett, 1978). It may also enhance or reduce the movement of water vapor across the shell (Meir and Nir, 1984). Ascorbic acid is a weak acid and the ability of diluted acid to interact with the eggshell cuticle was reported by Burley and Vadehra (1989). Egg injection or dipping with vitamins such as: pantothenic acid and AA has been applied during incubation to study their effects on hatchability of poultry eggs (Zakaria and El-Anezi, 1996 and Shafey, 2002). Hatchability improved by dipping or spraying of AA solutions (contains 10, 20, 30 and 40 gm. AsA/ liter distilled water) during the incubation period (Tag El-Din et al., 2004). Also, Awad and Abd Al-Haleem (2015) reported that hatchability (%) was significantly improved by about 2.64, 3.87, 8.77, 2.65 and 2.55 % for eggs dipped into 5.0 , 10.0 , 15.0 , 20.0 and 25.0 g AsA/ L solution as compared with those dipped into distilled water (0.0 g AsA/L), respectively. Our results agreed with Ipek et al. (2004) and Nowaczewski et al. (2012), who observed improvement in hatchability of eggs with injection of 3 and 6 mg of ascorbic acid. This fact shows that the effect of in-ovo vitamin C injection on the development of the embryo varies with solution concentration and the stage of embryo development in which the injection is performed.

Jochemsen and Jeurissen (2002) reported that the age at which the inoculation procedure is performed can affect the site where the product is applied. This improvement may be due to the decreasing of embryonic mortality where AsA as an anti-stress agent led to the reduction of corticosterone, which has a negative impact in collagen synthesis and the metabolism of minerals and vitamin D (Tullett, 1990; Kutlu, 2001; Lohakare et al., 2005 and Ghonim et al., 2008).

Hatchability percentage improved and embryonic mortality decreased by dipping eggs into AsA solution 10g/liter for up to 2 min before incubation period (Shafey, 2002), and by dipping or spraying AsA solutions at the 14th day of incubation (Ghonim et al., 2008). The improvement of hatchability may be due to the increase of embryonic viability, during the late part of incubation period, by the AsA dipping treatment which may act as a positive agent that led to the decrease of the excessive egg temperature as a result of intense fetal growth and increase metabolism (Shafey, 2002). Ohta et al. (1999) observed a reduction in hatchability after amino acid inoculation, in ovo, was performed before incubation. According to Ohta and Kidd (2001), product injection in ovo should be made either in the extra embryonic cavity or in the yolk sac to prevent hatching reduction; however, the authors injected at seven days of incubation and not pre-incubation as in the present study.

While, our results differ from those obtained by Pires et al. (2011), who observed an increase in hatching rate with injection of 1% ascorbic acid in ovo pre-incubation. Uni and Ferket (2003) reported that injecting high concentrations of the AsA may interfere with the osmotic balance and affect embryo development which indicates that the lower hatching rate was not due to the injected ascorbic acid changing the osmotic balance of the eggs excessively. Finally, Bhanja et al. (2007) studied the effect of in ovo injection of vitamins C on the embryonic and post-hatch growth performance. On the 14th day of incubation, five groups were injected with 50 mg Vitamin-C dissolved in 0.5 ml of sterile water. They found that chick weight to egg weight ratio (%) was higher ($P<0.01$) in chicks injected with Vitamin-C (72.26%) than un- injected control (70.94%). The higher in hatched chick'body weight my be due to increasing triiodothyronin hormone.

Blood parameters:

Hemoglobin (HGB) content values were significantly affected by the AsA injections. The results showed a linear effect on the levels of ascorbic acid in the solution injected in ovo prior to incubation. Our results were similar to Moura and Pedroso (2003) who reported that ascorbic acid is related to an increase of HGB values. Considering that hemoglobin is related to the transport of gases, these data suggest that increasing the concentration of the inoculated ascorbic acid solution increased respiratory rate, and consequently, the hematopoietic process and respiratory potential, without changing shell conductance. The increase of the HGB value might be associated with dehydration (Campbell, 1994). However, treatment did not affect either the weight of chicks at hatching or the presence of seemingly dehydrated chicks, thus ruling out this possibility. The other hematologic characteristics as hematocrit (HCT), red blood cells count (RBC), Mean corpuscular value (MCV) were not affected significantly ($p>0.05$) by the

treatments. These data are similar to those of Ghonim et al. (2009), who reported no effect of the injection of ascorbic acid on the erythrocyte of broiler chicks; however, the authors only looked at the effects of this vitamin in chicks resulting from eggs injected on the 14th day of incubation. In addition, Sgavioli et al. (2013) reported that injecting fertile eggs with ascorbic acid did not significantly affect some blood hematological parameters as red blood cell (RBC's) count, HCT %, MCV, or on serum glucose and triiodothyronin concentrations.

Although the egg is considered a functional food (Stadelman, 1999) and is an excellent source of protein, essential lipids, vitamins and minerals (Zeidler, 2002), many people reduce their consumption of eggs because they consider that high egg cholesterol content may cause cardiovascular diseases. Over the last three decades, many researchers have tried to reduce the egg cholesterol content by genetic selection, inclusion of drugs in the ration, or dietary manipulation of hens'diet (Shakeel, 2010). Many researches studied the effect of large doses of vitamin C (Ajakaiye et al., 2010; El-sheikh and Salama, 2010). However, the effect of vitamin C on yolk and serum cholesterol has not been established. Our results indicated that there were no significantly affect of in-ovo injection of ascorbic acid on serum cholesterol concentration. Our results are in agreement with Mohiti-Asli et al. (2007) who studied the effect of vit C (AsA) (200 mg / kg diet) on yolk and serum cholesterol. They found that there was no significant differences in serum and yolk cholesterol concentration between different experimental groups.

However, Sanda (2015) studied the effect of supplemention eighty (80) Isa brown layers of 28 weeks old with 0, 100, 150 and 200 mg vitamin C on yolk and serum cholesterol levels. They found that vitamin C reduced yolk and serum cholesterol levels.

IN CONCLUSION,

our study suggests injecting 50 microliters of 6 % ascorbic acid reduced embryonic mortality and improvd hatchability and hatched chicks'body weight.

Table (1): The effect of pre-incubation in ovo injection of ascorbic acid on hatchability, hatchability of fertile eggs, embryonic mortalities percentage and hatched chicks' body weight

Treatments	Hatchability (%)	hatchability of fertile eggs	Embryonic Mortalities (%)	hatched chicks' body weight (g)
Control	85.7^{d}	83.55^{d}	14.3^{a}	39.19^{b}
Ascorbic acid (2%)	89.7^{c}	86.73^{c}	10.3^{b}	38.23^{b}
Ascorbic acid (4%)	92.1^{b}	88.82^{b}	7.9^{c}	40.01^{b}
Ascorbic acid (6%)	94.8^{a}	91.28^{a}	5.2^{d}	46.32^{a}
SEM	0.05*	0.03*	0.001*	0.21*

Values are means ± standard error of the mean (SEM)
*Significant. N= 5 per replicate (15 per treatment)

Table (2): The effect of pre-incubation in-ovo injection of ascorbic acid on some blood hematological parameters of day old chicks.

Treatments	HCT(%)	HGB(g/dL)	RBC(10^6/mm^3)	MCV(µm3)
Control	33.05	12.09^{c}	2.74	120.62
Ascorbic acid (2%)	29.17	12.22^{c}	2.41	121.04
Ascorbic acid (4%)	28.64	13.93^{b}	2.46	116.42
Ascorbic acid (6%)	32.85	15.73^{a}	2.64	124.43
SEM	0.04	0.01 *	0.001	0.12

Values are means ± standard error of the mean (SEM).
* Significant. N= 5 per replicate (15 per treatment).
(HGB) Hemoglobin (HCT) Hematocrit
(RBC) Red blood cell counts (MCV) Mean corpuscular value

Table(3): The effect of pre-incubation in-ovo injection of ascorbic acid on serum cholesterol, glucose and triiodothyronin concentrations.

Treatments	Cholesterol (mm/mol)	Glucose (mg/dl)	T_3 (µ/L)
Control	9.18	167.22^{c}	98.8^{c}
Ascorbic acid (2%)	8.47	169.18^{c}	101.22^{c}
Ascorbic acid (4%)	8.77	172.29^{b}	112.44^{b}
Ascorbic acid (6%)	9.05	183.01^{a}	128.93^{a}
SEM	0.14	2.44*	6.55*

Values are means ± standard error of the mean (SEM)
* Significant. N= 5 per replicate (15 per treatment).
(T_3) Triiodothyronin hormone

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الملخص العربى

تأثير حقن فيتامين ج في بيض الهايلاين البياض قبل التفريخ على معدل الفقس و بعض صفات الدم فى الكتاكيت الفاقسة

امل محمد محمد بدران[1] – محمود حنفى ابو حطب[2] – نشات سعيد ابراهيم [2]
[1]معهد بحوث الانتاج الحيوانى – مركز البحوث الزراعية – وزارة الزراعة
[2]قسم التطبيقات البيولوجية – مركز البحوث النووية – هيئة الطاقة الذرية

هدف هذا البحث هو دراسة تأثير حقن بيض التفريخ لسلالة الهايلاين البياض قبل التفريخ بفيتامين ج و دراسة تأثير ذلك على معدل الفقس و بعض صفات الدم. تم تنظيف البيض قبل التفريخ و عدده 300 بيضه بالكحول و تم وزن البيض و تقسيم البيض عشوائيا الي اربعة مجموعات تضم كل مجموعة خمسة و سبعون بيضة كما تم تقسيم كل مجموعة الى ثلاثة مكررات تضم كل مكرر خمسة و عشرون بيضة حيث تم حقن المجموعة الاولى بخمسون ميكروليتر من الماء المقطر فى الغرفة الهوائية و تم اعتبارها المجموعة المقارنة (كونترول). بينما تم حقن المجموعة الثانية و الثالثة و الرابعة بالغرفة الهوائية بفيتامين ج مذاب فى خمسون ميكروليتر من الماء المقطر بمعدل 2 و 4 و 6 % على التوالى. تم تحضين البيض على 37.5 درجة مئوية و 60 % رطوبة نسبية مع التقليب الاتوماتيكى مرة كل ساعتين خلال اول تسعة عشر يوما من التفريخ و حتى نقل البيض الى المفقس حيث يتوقف التقليب و ترفع الرطوبة النسبية الى 70 %. فى اليوم العاشر من التفريخ تم فحص البيض ضوشيا للاستبعاد البيض الغير مخصب . و قد دلت النتائج على انه بالرغم من الارتفاع المعنوى لنسبة الفقس و وزن الجسم للكتاكيت الفاقسة فى المجموعات المعاملة بفيتامين ج مقارنة بالكونترول الا ان نسبة الفقس كانت مرتفعة و اكثر معنوية فى المجموعة المحقونة بمعدل 6 % من فيتامين ج عن الكونترول او كلا المجموعتين الاخريتين (2 و 4 % من فيتامين ج) و كانت هذه النتائج علي العكس تماما من نتائج نسبة النفوق.كما لم يكن هناك اى فروق معنوية بين المجموعات المعاملة بفيتامين ج بمعدل 2 % مقارنة بالكونترول فى نسبة الهيموجلوبين الا انها كانت مرتفعة معنويا فى المجموعة المحقونة بمعدل 4 % و كانت اكثر ارتفاعا بصورة معنوية عند الحقن بمعدل 6 % من فيتامين ج عن كلا المجموعتين الاخريتين (2 و 4 %). بالرغم من انه لم يكن هناك اى فروق معنوية بين المجموعات المعاملة بفيتامين ج مقارنة بالكونترول فى محتوى سيرم الدم من الكوليستيرول الا ان محتوى سيرم الدم من كل من الجلوكوز و هرمون الدرقية كانت مرتفعة معنويا فى المجموعة المحقونة معدل 4 % و كانت اكثر ارتفاعا بصورة معنوية عند الحقن بمعدل 6 % عن كلا المجموعتين الاخريتين (2 و 4 %).
و توصى الدراسة بحقن فيتامين ج بتركيز 6 % قبل التفريخ لتحسين الفقس و خفض معدل النفوق الجنينى و زيادة وزن الكتاكيت عند الفقس.