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Research Article

Effects of Antioxidants Via In Ovo Injection on Chick Development and Developmental Stability of Bilateral Morphological Traits Induced Maternal Hypoxia in Daily Hatched Quail Chicks

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Abstract

The study aimed to investigate effects of antioxidants via in ovo (IO) injection, one of the applications based on maternal effects, to different sacs of embryonated eggs obtained from quail breeders laid at high altitude on chick development and developmental stability of bilateral morphological characteristics. A total of 180 incubating eggs obtained for 7 days from 40 females at 120 days-old at a 1720 m-altitude were randomly divided into 3 groups as control (CONT), IO alpha tocopherol (α-TO) and IO ascorbic acid (AA). Sixty eggs of the control was non- injection. The rest of 120 eggs (60 eggs/group) assigned to injection groups were injected into yolk sac a 25 µl vitamin E solution containing 3.75 mg α-TO by 120th hour of incubation, and amniotic sac a 25 µl solution containing 2.5 mg AA by 360th hour of incubation. At hatch, chick weight, organ weights, and chick length, bilaterally lengths of face, middle toe and shank were measured. Relative asymmetry (RA) of these traits were calculated. The effect of IO administration is insignificant on the lengths of chick, face and middle toe and weights of chick, and relative liver, heart and brain. Whereas the shank length increased in the AA group, it decreased in the α-TO group. The RA of middle toe length was higher in the AA group and was lower in the α-TO group than CONT. The RA shank length was found the highest in the AA group differently from other groups. The mean RA of examined lengths was the highest in the AA group and was the lowest in the α-TO group. The yolk sac weight was significantly lower in the CONT and α-TO groups than AA group. Proventriculus + gizzard weight was the lowest in the AA group and was the highest in the α-TO group. Injected antioxidants to embryonic sacs as a tool to eliminate the adverse effects of hypoxic-stress on several developmental traits and developmental stabiliy of bilateral morphological traits can be resulted in antioxidant-specific in day-old quail chicks.

Key words: α-tocopherol, ascorbic acid, high altitude, hypoxia, ın ovo enjection.

Günlük Bıldırcın Civcivlerinde Maternal Hipoksiyanın Uyardığı Çift Yönlü Morfolojik Özelliklerin Gelişme Stabilitesi ve Civciv Gelişimi Üzerine İn Ovo Enjeksiyon Aracılığı ile Antioksidanların Etkileri

Özet

Bu çalışma, yüksek rakımda yumurtlayan damızlık bıldırcınlara ait kuluçkalanan yumurtaların farklı embriyonik keselere, maternal etkilere dayalı uygulamalardan biri olan in ovo (IO) aracılığı ile enjekte edilen antioksidanların civciv gelişimi ve çift yönlü morfolojik özelliklerin gelişim stabilitesi üzerindeki etkilerini araştırmayı amaçlamıştır. Yüksek rakımda (1720 m) 120 günlük yaştaki 40 damızlık bıldırcından 7 gün boyunca elde edilen toplam 180 adet kuluçkalık yumurta rasgele kontrol (KONT), IO alfa tokoferol (α-TO) ve IO askorbik asid (AA) olacak şekilde 3 gruba ayrılmıştır. Kontrol grubu için 60 adet yumurtaya enjeksiyon işlemi uygulanmamıştır. Enjeksiyon gruplarına aktarılan 120 yumurtanın ilk 60 adedine kuluçkanın 120. saatinde yumurta başına 3.75 mg α-TO olacak şekilde 25 µl vitamin E içeren bir solüsyon sarı keseye; geriye kalan 60 adet yumurtaya kuluçkanın 360. saatinde yumurta başına 2.5 mg AA olacak şekilde 25 µl AA içeren bir solüsyon embriyonun amniyon kesesine enjekte edilmiştir. Çıkışta, civciv ağırlığı, organ ağırlıkları ile civciv uzunluğu, sağ

ve sol olacak şekilde yüz, orta parmak ve incik uzunlukları ölçülmüştür. Bu çift yanlı özelliklere ait oransal asimetri (OA) değerleri hesaplanmıştır. IO uygulamanın civciv, yüz ve orta parmak uzunluğu ve civciv ağırlığı, oransal karaciğer, kalp ve beyin ağırlıkları üzerine etkisi önemsiz saptanmıştır. İncik uzunluğu AA grupta artarken, α-TO grupta azalmıştır. KONT gruba göre orta parmak uzunluğunun OA değeri AA grupta yüksek, α-TO grupta düşük bulunmuştur. İncik uzunluğunun OA değeri AA grupta diğer gruplardan farklı olarak en yüksek düzeyde elde edilmiştir. İncelenen uzunlukların ortalama OA değeri AA grupta KONT'den daha yüksek, α-TO grupta daha düşük saptanmıştır. Sarı kese ağırlığı AA gruba göre KONT ve α-TO gruplarda önemli düzeyde düşük saptanmıştır. Proventrikulus + taşlık ağırlığı AA grupta düşük, α-TO grupta daha yüksek bulunmuştur. Günlük yaştaki bıldırcın civcivlerinin bazı gelişim özellikleri ile birlikte çift yanlı morfolojik özelliklerin gelişim stabilitesi üzerine hipoksiyaya bağlı stresin olumsuz etkilerini ortadan kaldırmak için bir yöntem olarak kullanılan embriyonik keselere antioksidanların enjeksiyonu antioksidanın çeşidine bağlı olarak etkiler ortaya koyabilmektedir.

Anahtar kelimeler: α-tokoferol, askorbik asit, yüksek rakım, hipksiya, in ovo enjeksion.

Introduction

Oxygen is a critical participant in the formation of reactive oxygen species (ROS) or free radicals and provides continuity for many biochemical and metabolic processes in cellular (Giordano, 2005). ROS and free radicals are often produced by the oxygen (O_2) metabolism. If a free radical is formed and not decreased by antioxidant protective mechanism, tissue damages can occur by chemical radicals and ROS. This causes to oxidative stress (Bottje and Wideman, 1995). Furthermore, hypoxic stress, which is due to high altitude, or lower O_2 concentration than %21 O_2 concentration, reduce embryonic development and occur negative effects such as pulmonary hypertension or ascites susceptibility, and failures on development of cardio-vascular system (Bottje and Wideman, 1995; Wideman et al., 2013; Khajali and Wideman, 2016). These negative effects during embryo development occur, especially when incubated embryonated hatching eggs at high altitude (Giussani et al., 2007; Babacanoğlu and Güler, 2018), or at ≤%17 O_2 concentration (Lourens et al., 2007).

Antioxidant protective mechanisms are improved to deal with ROS of living organisms and to minimize stress emerged due to oxygen metabolism at adverse environmental conditions (Halliwell and Gutteridge, 1999). For this reason, the level of O_2 must be at a certain level in the sense of the presence of natural antioxidants that ensure to survive of the living organism (Halliwell, 1994). α-tocopherol (α-TO) including the most active isomer of Vitamin E is a non-enzymatic lipophilic antioxidant, and ascorbic acid (AA) is a non-enzymatic hydrophilic antioxidant. AA works by restoring the antioxidant properties of tocopherols (Cotella et al., 2003). Freshly laid eggs is not contain AA that is synthesized in the embryo by means of egg yolk sac membrane at the first stages of embryo development (Yew, 1985). AA reacts directly with O_2 to combat free radicals (Surai, 2002) by its protective trait against many free radicals and act synergistically with tocopherol to regenerate the tocopherol radicals (Carr and Frei, 1999).

Antioxidants within protective antioxidant mechanism divided into two general groups; lipid- and water-soluble antioxidants. Egg yolk has lipid-soluble antioxidants and albumen contains water-soluble antioxidants originated from maternal. The efficiency of the antioxidants is possible to determine with more effective some application based on maternal effects (Babacanoğlu et al., 2018). For example, it has been reported that in ovo (IO) injection, an application for the detection of maternal effects, into egg yolk prior to incubation, or into yolk sac of embryo during incubation is more effective and quicker than any application in response to egg yolk or albumen from maternal (Hossain et al., 1998; Uni et al,, 2005).

Asymmetry in bilateral morphological traits is pointed as a potential animal welfare indicator (Knierim, 2007) because it reflects the ability of an individual to cope with stress factors, such as chronic hypoxic conditions that may affect it's the morphological characteristics during critical periods of embryo development (Dzialowski et al., 2002). Therefore, this study was aimed to evaluate effects of IO α-TO injection into yolk sac and IO AA injection into amniotic sac of embryonated egg on chick development and lengths of morphological traits and RA of bilateral morphological traits in newly hatched quail chicks at a 1720 m altitude.

Material and Method

All procedures were approved by YYU Animal Care and Use Committee (permit number 2016-05).

Animal material

Fourty female and 8 male Japanese quail breeders (*Coturnix coturnix japonica*) as animal material were used reared under similar environmental conditions. Each of female quail breeders in first year of laying were inseminated with 0.020 cc ejaculate. After the third day of inseminated, the incubating eggs were collected for

7 days. A total of 180 incubating eggs obtained from females were divided into 3 groups including 60 eggs as 4 replicates in each group which were incubated. Prior to incubation, the average egg weight was 12.61 g.

Experimental design

A group was non-injected as control (CONT), alpha tocopherol (α-TO) group: a 25 µl vitamin E solution containing 3.75 mg α-TO (Evin I.M. dl-α-tocopherol acetate sterile solution) was injected into the yolk sac of each egg on day 5 of embryonic age (120th hour of incubation); and ascorbic acid (AA) group: a 25 µl solution containing 2.5 mg AA (Redox-C®) to amniotic sac of each egg was injected on day 15 of embryonic age (360th hour of incubation) using in ovo method. After sterilized of the large end of each egg (injection site) with 70 % ethanol, IO injection to the embryonic sacs was made by used a 0.5-ml disposable syringe and a 26-gauge, 13-mm needle. Needle punctures in the eggshell were sealed immediately with paraffin. After injection, the eggs were returned to the incubator. A single stage incubator was set at 37.7 °C, 60% relative humidity and 90° turning per 2 hours during 372 hours of incubation and at 37.2 °C and 70 % relative humidity from 372 hours to 420 hours of incubation.

Examined traits

At hatch, a total of 36 chicks, comprising 3 randomly selected chicks from each replicate of the groups were removed from the hatcher. Chick length (cm) was measured using a digital caliper (±0.1 mm). Both sides (left and right) of bilaterally morphological traits (face, middle toe and shank lengths) were also measured three times for each chick (Hill, 2001; Babacanoğlu et al., 2018). Face length was measured from ear hole to the merged point of the beak. Middle toe length was measured from the beginning of the third toe to the tip of the nail on the third toe. Shank length was measured from the top of the tarsometatarsus to the tip of the nail on the third toe. Developmental instability is defined as deviations from symmetrical growth on the right and left sides for any bilateral trait (Yang et al., 1997). Relative asymmetry (RA), which is the absolute value of asymmetry of the left (L) minus the right (R) divided by the size of the mean L and R lengths of face, middle toe and shank, was determined using the following formula Eq. : $RA = \{(L-R)/[(L+R)/2]\} \times 100$ (Yang et al., 1997). Mean RA was calculated as the average RA of the lengths of the face, middle toe and shank. Chick weight were determined individually for the same chicks. After the chicks were killed by cervical dislocation, the RYS, heart, liver, gizzard + proventriculus after emptying and brain were excised from each chick and weighed. Relative weights of the organs and RYS were shown as percentages of the absolute weight of the chick.

Statistical analysis

For the statistical analysis of the obtained data, IO administration groups were subjected to ANOVA with the GLM procedure of the JMP statistics package (SAS Institute Inc., 2007). Tukey HSD test was used to compare the mean of the groups.

Results and Discussion

The effect of IO administration on the lengths of chick, face and middle toe is insignificant (Table 1). Similarly, RA value of the face length did not differ in the non-injected group and injected groups. IO administration significantly affected shank length. The highest shank length was found in the AA group, whereas the lowest shank length was found in the α-TO group, but both of injected groups were not differ from CONT group (Table 1). The RA of middle toe length was significantly different between IO groups and CONT group. The RA of middle toe length was higher for the AA group and lower for the α-TO group than CONT group (Table 1). The RA of the shank length was the highest in the AA group differently comparative with other groups. The RA of shank length in the α-TO group was found in higher than the RA of the shank length from chicks of the control, but α-TO group was lower than AA group (Table 1). The mean RA of face, middle toe and shank lengths was the highest in the AA group and the lowest in the α-TO group, which was significantly the different between IO treatment groups, compared to the CONT (Table 1). The yolk sac weight was significantly lower in the CONT and α-TO groups than AA group (Table 2). Proventriculus + gizzard weight was significantly the lowest in the AA group and the highest in the α-TO group, but these groups were not different from the CONT group (Table 2). There was no a significant difference on chick weight and relative liver, heart, and brain weights between the IO treatment groups and the CONT group (Table 2).

Table 1. Effects of IO administration on lengths of chick and bilateral traits, and relative asymmetry for lengths of bilateral traits induced hypoxia at high altitude at newly hatched chicks.

	Lengths of bilateral traits				Relative asymmetry (RA) for lengths of bilateral traits			
	Chick length	Face	Middle toe	Shank	Face	Middle toe	Shank	Mean RA
	cm		mm					
IO administration								
CONT	11.61	8.36	11.16	10.09^{ab}	0.06	0.58^{b}	0.02^{c}	0.24^{b}
α-TO	12.09	8.06	12.39	9.51^{b}	0.04	0.03^{c}	0.11^{b}	0.06^{c}
AA	11.62	8.29	11.81	11.23^{a}	0.21	1.39^{a}	1.17^{a}	0.93^{a}
SEM	0.16	0.29	0.38	0.39	0.07	0.02	0.01	0.03
ANOVA								
P value	0.093	0.766	0.094	0.032	0.149	0.048	0.014	<0.001

CONT: Control; α-TO: In ovo α-tochopherol injected group; AA: In ovo ascorbic acid injected group.
SEM: Standard error mean.

Table 2. The effect of IO administration on development induced hypoxia at high altitude at newly hatched chicks.

	Chick weight	Yolk sac weight	Liver weight	Heart weight	Proventriculus+gizzard weight	Brain weight
	g			%		
IO administration						
CONT	8.61	8.22^{b}	3.01	1.17	5.58^{ab}	3.27
α-TO	8.48	7.26^{b}	2.78	0.99	6.07^{a}	3.33
AA	8.15	12.38^{a}	2.65	1.07	5.29^{b}	3.24
SEM	0.22	1.23	0.14	0.06	0.06	0.18
ANOVA						
P value	0.329	0.015	0.204	0.173	0.047	0.938

^{a,b,c} Means within a column with different superscript differ significantly at P<0.05.
CONT: Control; α-TO: In ovo α-tochopherol injected group; AA: In ovo ascorbic acid injected group.
SEM: Standard error mean.

The hypothesis of this study was to obtain optimum embryo development by means of IO injected antioxidants to different embryonic sacs to mitigate negative effects of hypoxia as well as maternal hypoxic stress due to a 1720 m-altitude on chick development and developmental stability during pre-hatch. The effect of IO administration on the lengths of chick, face and middle toe is insignificant. Similarly, RA value of the face length did not differ in the non-injected group and the injected groups in the study. However, Babacanoğlu et al. (2018) concluded that chick and middle toe were the longest following IO α-tocopherol administration on day 5 of embryo development, the results of these studies indicated that IO α-tocopherol administration had positive effects on chick quality. The highest RA of shank and middle toe lengths determined in the AA group was caused to the increased mean RA of bilateral morphological lengths. The conclusions suggested that the daily chicks from AA injected to amniotic sac of the embryonated egg under hypoxic stress during embryonic development as well as maternal hypoxic stress may have not challenge with both of stress conditions during hatching. The highest RA from broilers with maternal stress had obtained that indicated long-term effect of maternal stress (Babacanoğlu and Yalçın, 2015). Moreover, the longest shank length observed following IO AA injection indicated that the newly hatched chicks from this injection group cannot cope with stress, which the highest IR-Ll for the shank length was observed in the effect of the same hypoxic-stress without any manipulated incubation condition in broilers as reported by Babacanoğlu and Güler (2018).

In this study, IO injected antioxidants at hypoxic-high altitude did not affect chick development due to unchanged chick weight and relative liver, heart, and brain weights. Hypoxia is defined as a typical stress caused by the fall in barometric pressure with increasing altitude (Beall, 2006). Actually, maternal hypoxia is also a stress factor determinant due to the effect on oxygen

metabolism. However, Babacanoğlu and Yalçın (2014) resulted that maternal stress did not adverse effect on chick weight in broilers. The efficiency of the transfer to egg of antioxidants originated from maternal sources is an important factor in embryonic development in poultry (Babacanoğlu and Özelçam, 2013). Antioxidants concentrations in the tissues transferred from yolk sac, especially in the liver of newly hatched chicks are responsible for normal embryonic/chick development (Surai, 1999) so antioxidant protection is an important mechanism on chick development at hatching time (Surai, 2002). In the current study, the highest the residual yolk sac weight and the lowest proventriculus + gizzard weight were obtained by IO AA injection to amniotic sac, shown that chicks were less nutrient-absorbing from residual yolk sac as a sign of the effect of hypoxic-stress, though there was no a negative effect on chick weight. Similarly, Zhang et al. (2018) had reported that effect of IO administration of different AA dosages to broiler hatching eggs did not eliminate the negative effects of a 48-hour post-hatch holding time on yolk sac utilization. The results of this study indicated that the day-old quail chicks from AA, which was an important energy source, were the more less utilization of nutrients from yolk sac and did not improve digestive system efficiency.

When compared with IO injected AA, IO injected α-TO led to higher utilization from the yolk sac of embryo and digestive system efficiency of the day-old quail chick's that may be related to dependence of α-TO concentration in the embryonic tissues (Surai et al., 1996; 1999). As a result, increased α-TO concentration in the egg yolk by IO at day 5 of incubation is an effective way to protect quail chicks from oxygen stress during hatching. It might be resulted in increased levels of vitamin E isomers of tissues by IO α-TO (Babacanoğlu et al., 2018) and vitamin E level in the liver of day-old chicks (Surai, 2002). This phenomenon means that IO antioxidant to yolk sac can be used as a tool to protect quails from stress conditions during hatching. Also, the α-TO treatment also could be beneficial for the development of digestive systems of the day-old chicks (Surai et al., 1996). Another explanation may be related to injected antioxidants concentration in the tissues on development of digestive systems of the day-old chicks because yolk sac membrane contains comparatively high concentrations of α-TO, but low levels of AA during the second half of incubation (Surai et al., 1996), whereas oxygen concentration in the tissues is higher by increased metabolic activity and tocopherol concentration is decreased in the tissues of newly hatched chick (Surai, 2002).

Conclusion

In the light of the results of this study is clearly indicated that two main antioxidants of injection to embryonic sacs had difference effects on yolk sac absorption and digestive system development without chick development. Also, the developmental stability of any bilateral morphological trait may be affected antioxidant-specific in newly hatched quail chicks.

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