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Effects of In Ovo Pollen Extract Injection to Fertile Broiler Eggs on Hatchability and Subsequent Chick Weight

Isa COŞKUN[a]*, Huseyin ÇAYAN[a], Ömer YILMAZ[b], Atilla TASKIN[a], Emre TAHTABİÇEN[c], Hasan Ersin SAMLİ[c]

[a]Department of Animal Science, Faculty of Agriculture, Ahi Evran University, 40100 Kirsehir
[b]Apiculture Research Station Directorate, 5200 Ordu
[c]Department of Animal Science, Faculty of Agriculture, Namik Kemal University, 5900 Tekirdag

*Corresponding author: isa.coskun@ahievran.edu.tr

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Abstract

The aim of this study was to determine the effect of pollen extract injection into the amnion of fertile broilers eggs on hatchability and relative chick weight to egg weight. Eggs were provided from 34 wk old Ross 308 parent stocks. In the trial 180 fertile broiler eggs were used. Three groups (control, negative control and pollen extract injected group) with three replicate were also included. Injections carried out at the 16th day of incubation. At the end of the study, hatchability was founded at control; negative control and pollen extract injected groups 89.1%, 82.3% and 73.1%, respectively. There were no statistically difference between control and pollen extract injected groups in terms of hatchability, but hatchability decreased in negative control group (P<0,05). Chick weight/initial egg weight after hatch were founded 70.1%, 71.1%, 73.5% at control; negative control and pollen extract injected groups, respectively. It was determined that injection of pollen extract to amnion of eggs statistically increased chick weight/initial egg weight according to the control and the negative control groups (P<0,05). At the end of the study, it was concluded that pollen extracts might be used as a feed ingredient to obtain heavier chicks after hatch, but different extraction methods or different pollen extracts need to be researched.

Keywords: in ovo injection, pollen extract, broiler, eggs, hatchability, subsequent chick weight

Döllü Etlik Piliç Yumurtalarına Polen Ekstraktı Enjeksiyonunun Kuluçka Randımanı ve Civciv Ağırlığı Üzerine Etkileri

Özet

Bu çalışmanın amacı döllü etlik piliç yumurtalarının amniyotik sıvılarına polen ekstraktı enjeksiyonunun kuluçka randımanı ve yumurta ağırlığına göre oransal civciv ağırlığını belirlemektir. Döllü etlik piliç yumurtaları 34 haftalık yaştaki damızlık Ross 308 sürüsünden elde edilmiştir. Çalışmada 180 adet döllü etlik piliç yumurtası kullanılmıştır. Çalışma 3 muamele grubu (Kontrol, Negatif kontrol ve Polen Ekstraktı) ve her muamele grubuna ait 3 tekerrürden oluşturulmuştur. Enjeksiyonlar kuluçkanın 16. gününde gerçekleştirilmiştir. Çalışmanın sonunda kuluçka randımanları kontrol, polen ekstraktı ve negatif kontrol gruplarında sırasıyla %89.1, %82.3, %73.1 olarak bulunmuştur. Kuluçka randımanı bakımından polen ekstraktı enjeksiyonu ile kontrol grubu arasında istatistiki farklılık oluşmazken, negatif kontrol grubunda kuluçka randımanı düşmüştür (P<0,05). Ayrıca başlangıç yumurta ağırlığına göre % civciv ağırlıkları da kontrol, negatif kontrol ve polen ekstraktı enjekte edilen gruplarda sırasıyla %70.1, %71.1, %73.5 olarak bulunmuştur. Polen ekstraktı enjeksiyonunun % civciv ağırlığını kontrol ve negatif kontrol gruplarına göre istatistiki olarak arttırdığı belirlenmiştir (P<0,05). Araştırma sonunda polen ekstraktının kuluçkadan sonra daha ağır civciv elde etmek için in ovo besin maddesi olarak kullanılabileceği, farklı ekstraksiyon metotlarının ya da farklı polen çeşitlerinin de araştırılması gerektiği sonucuna varılmıştır.

Anahtar kelimeler: in ovo enjeksiyon, polen ekstraktı, etlik piliç, yumurta, kuluçka randımanı, civciv ağırlığı

Introduction

The aim of in ovo feeding is to increase embryo weight and immunity at hatch. Embryo consumes the nutrients in eggs towards the end of the incubation and begins to use body reserves for emergence (Ferket, 2006). Therefore, chicks may lose weight in this period. It has reported that embryo weight may be increased by different in ovo nutrient supplementation during the last period of incubation (Ferket, 2006). But the most important problem in in-ovo feeding studies is hatchability. Because decreasing hatchability is undesirable situation for commercial broiler hatcheries. Therefore, it is important to feed embryo without reducing hatchability at in ovo injection studies. In literature, there are different results about the effects of different nutrient injection to fertile eggs on hatchability, but most of the results were trend to decline. For example Ohta and Kidd (2001) have reported that injection of different amino acid solutions into the 13 mm depth of eggs was not affected hatchability rate, but injection to 19 mm decreased hatchability at 7th the day of incubation. Zhai et al. (2008) have found that injection of L-Carnitine to 2.54 cm depth of fertile eggs at the 18th day of incubation decreased hatchability although there was no statistically significance. At the other study of Zhai et al. (2011) injection of carbohydrates and dextrin into 2.49 cm depth of eggs at 18.5th day of incubation decreased hatchability statistically, but carbohydrates and dextrin enhanced subsequent chick weight (SCW) about 4-5%, especially glucose injection increased SCW from 72% to 76% at emergence. Although, it has been reported that many nutrients provide weight gain of embryos, the investigation of new nutrients for in-ovo feeding is important. To determine the effects of different nutrients on embryo growth and hatchability are the future of in ovo feeding studies. In spite of pollen extracts have rich nutrient content, the effects of in ovo feeding of pollen extracts on chick quality and hatchability is not yet determined. Wang et al. (2007) have stated that pollen supplementation of broiler feed provided to increase of villus length of duodenum (37.1%), jejunum (28.1%) and ileum (18.6%). Wang et al. (2007) have reported that pollen may be used as a feed additive for broiler ration because of pollen increased early development of the gastrointestinal tract of broilers. Also, Attia et al. (2004) have reported that supplementation of bee pollen to broiler diet increased live weight gain of broiler chicken at 35th d of study and improved the feed conversion ratio. The aim of this study was to determine the effect of in ovo pollen extract injection to fertile broiler eggs on hatchability and subsequent chick weight.

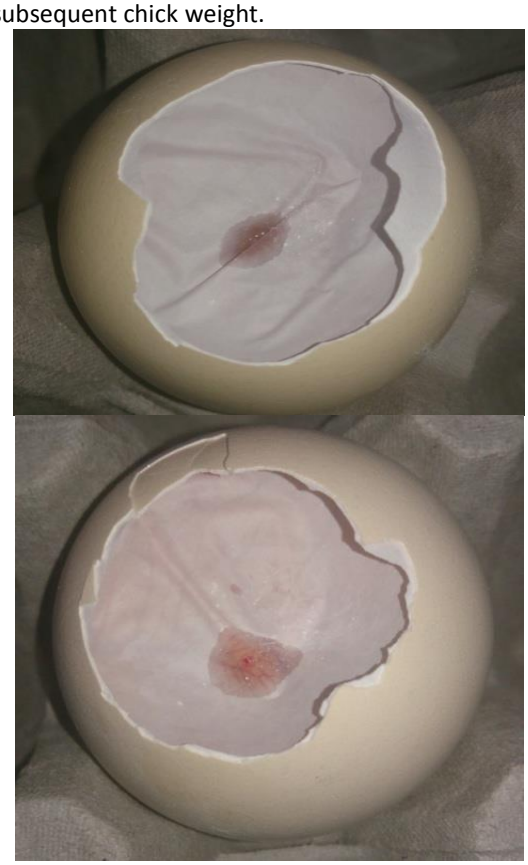

Figure 1. Injection sites of the fertile eggs 16th day of incubation.

Materials and Methods

This study was approved by the Ethical Committee of Ahi Evran University (AEÜ.HADYEK). In the trial, fertile eggs provided from a breeder flock at 34 wk of age (Ross 308) were obtained from Bakpilic (Bakpiliç Entegre Tavukçuluk AŞ, Turkey) and eggs were weighed, numbered and incubated at 37.8°C and 56% relative humidity in a Cimuka incubator (Cimuka, Turkey). At the 12th day of incubation, eggs were tested with lamp and those unfertilized or with dead embryos were discarded. The remaining eggs (180) were divided into 3 treatment groups with equal standard of three replicate and put in the incubator. Two eggs from each replicate opened and illustrated to determine the accuracy of the injection site during injection (Figure 1). The experimental groups were: 1) Control (no injection) 2) Negative control (because of pollen extract was produced with coca solution, coca solution was used as negative control group) and 3) Pollen extract injection. Eggs were distributed according to the distribution of frequency equal weight to each replicate. During injection, the blunt side of the egg was sterilized with 80% ethanol. In ovo administration of pollen extract at 1 ml per egg was applied through 18 mm

deep of the blunt side of the eggs by using a 21-gauge needle. Before application, we calculated average membrane distance as 18 mm at 16th day of incubation via light control. Thus, injection depth was chosen as 18 mm both to make absolute injection into amniotic fluid and to avoid damaging embryo. Post hatch weight of chicks determined 6 hours after hatch by using electronic scale (0.01 g). Also, hatchability and subsequent chick weights (Post hatch chick weight/ initial egg weight) determined.

Preparation of Pollen Extract and Coca Solution

Pollen extract was prepared in Ordu Apiculture Research Station Directorate. Pollen collected from Amasya province at 2013, pollens is dried in dark room condition and collected in glass bottle at -18 degree in deepfreeze. Pollen structure is formed by sunflower and season flowers pollens. The extraction method which was applied by Aytug et al. (1991) was used for preparing the extracts of collected pollen, as an extractive Coca solution and for sterilization sterile filtration technique was used. 500 mg Pollen and 4.5 ml coca solution was added in falcon tube and mixed 24 hours +4 ˚C degree in magnetic stirrer. Pollen extract centrifuged (2750 rpm) and filtered 8 times for avoid of solid residues. Coca solution is formed from NaCl (9 gr), $NaHCO_3$ (3 gr), C_6H_5OH (5 gr) and distilled water (983 ml). Coca solution pH was balanced to 8.2 with a few drops of 10% NaOH (Aytug et al., 1991).

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) by SPSS 17.0 for Windows (SPSS Inc., Chicago, IL). Duncan's test was used to determine the effect of treatments and differences which were considered to be significant at $P < 0.05$.

Table 1. Effects of in ovo pollen extract injection to fertile broiler eggs on hatchability and chick weight**.**

	Hacthability (%)	Subsequent Chick Weight (%)
Control	89.1[a]	70.1[b]
Coca Solution (as Negative Control)	73.1[b]	71.1[b]
Pollen Extract	82.3[a]	73.5[a]
SEM	2.64	0.54
P Values	0.013	0.004

[a,b] Numbers within a column lacking a common superscript differ (P<0.05)
SEM= Standart Error of Means

Result and Discussion

The results of the study showed that in ovo pollen extract injection (PEI) at the 16th day of incubation into the amniotic fluid of fertile broiler eggs increased subsequent chick weight (SCW) from 70.1% to 73.5% among the control and PEI groups, respectively (Table 1) and this differentiation was statistically significant (P<0.05).

The yolk nutrients are essential not only for the development of body and gastro intestinal tract but also for the maintenance of the embryo mobility which requires energy during late-term incubation (Christensen et al., 2000). Also, Chen et al. (2009) have reported that high level of energy needs for the rapid development of the digestive organs and to provide development of embryos at the last day of incubation. It has reported that poultry embryos use to degradation of proteins from pectoral muscle to provide the energy in late term incubation via gluconeogenesis (Hammer and Dikson, 1989), as a result of protein degradation to provide energy from pectoral muscle decreases breast meat weight in chicks (Vieira and Moran, 1999). Ferket, (2006) has reported that embryo naturally consume supplemental nutrients orally before emergence and chick weight increase with injection an isotonic in ovo feeding solution into the amniotic fluid. Increasing of chick weight about 4.85% according to control group with pollen extract injection to fertile broiler eggs may be due to rich nutrient content of pollen extract using in this trial. At the 16th day of incubation, injection of pollen extract to amniotic fluid has showed that embryos consumed pollen extract. Cabi (2005) has reported that pollen extract producing by coca solution have all amino acids and plant growth factors like gibberellins, cytokines, auxin and brassinolide. Also, Erdogan and Dodologlu (2005) have reported that pollen include all essential and non essential amino acids and are the unique nutrients for bees. In the literature, study or studies that determine the effects of pollen extracts on in ovo feeding are not available. As well as studies about the pollen and pollen extracts addition to broiler feed on broiler performance is not sufficient. Wang et al. (2007) have reported

that inclusion of pollen to broiler feed increased thickness and length of gastro intestinal tract and increased length of the villi in duodenum, jejunum and ileum, respectively. Wang et al. (2007) have demonstrated that pollen may promote early development of gastro intestine tract of broilers and pollen may be food supplement for broilers.

Hatchability was 89.1%, 82.3% and 73.1% among the control, PEI and negative control groups, respectively. In the present study, pollen extract injection into the amnion did not affect hatchability, but coca solution injection decreased hatchability than those of control and pollen extract injected groups. It has reported that injection time and the injection depth profoundly effects hatchability. According to the results of previous studies, at the first days of incubation (Kocamis et al., 1998; Salmanzadeh et al., 2011, 2012; Ohta and Kidd, 2001; Ohta et al., 1999) and the last days of incubation (Zhai et al., 2008, 2011; Keralapurath et al., 2010; Mcgruder et al., 2011; Bello et al., 2013; Coskun et al., 2014) decreased hatchability when injection of different nutrients into the amnion with different injection depth. However, there are studies indicating that in ovo injection did not affect hatchability (Uni et al., 2005; Foye et al., 2007; Ipek et al., 2004; Bottje et al., 2010; Chamani et al., 2012). Besides nutrients for in ovo injection is an important factor for embryonic development and hatchability. Chamani et al. (2012) have reported that injection of albumin and dextrose mix increased body weight of embryos and have any negative effect on hatchability. In conclusion, injection of pollen extract at the 16th day of incubation to fertile broiler eggs increased subsequent chick weight about 4.85% from control group and not affected hatchability. According to the this results, pollen extract may be used as a feed ingredient to obtain of heavier chicks after hatch, but different pollen extracts or different extraction methods of pollen extracts need to be examined for in ovo feeding studies.

References

Attiaa, Y. A., Abd Al-Hamid, A. E., Ibrahim, M. S., Al-Harthi, M. A., Bovera, F., Elnaggar A.Sh., 2014. Productive performance, biochemical and hematological traits of broiler chickens supplemented with propolis, bee pollen, and mannan oligosaccharides continuously or intermittently. Livestock Science 164 87-95.

Aytug, B., Dal, M., Colakoglu, B., Oner, A., Peremeci, E., Temiz, D., Guvener, B., Buyukdevrim, S., Guven K.C., 1991. "Türkiye Alerjik Polenlerinden Polen Ekstresi Hazırlanması ve Deri Testi Uygulamaları", Acta Pharmaceutica Turtica, 33, 85-95.

Bello, A., Zhai ,W., Gerard, P. D., Peebles E.D., 2013. Effects of the commercial in ovo injection of 25- hydroxycholecalciferol on the hatchability and hatching chick quality of broilers. Poultry Science, 92, 2551–2559.

Bottje, W., Wolfenden, A., Ding, L., Wolfenden, R., Morgan, M., Pumford, N., Lassiter, K., Duncan, G., Smith, T., Slagle, T., Hargis, B., 2010. Improved hatchability and posthatch performance in turkey poults receiving a dextrin-iodinated casein solution in ovo. Poultry Science, 89, 2646-2650.

Chamani, M., Tasharrofi, S., Forudi, F., Sadeghi, A. A., Aminafshar, M., 2012. Evaluation the Effects of In-ovo Injection of Different Nutrients on Hatch Percentage, Performance and Carcass Parameters of Broilers. Annals of Biological Research, 3 (7), 3771-3776.

Chen, W., Wang, R., Wan, H. F., Xiong, X. L., Peng, P., Peng, J., 2009. Influence of in ovo injection of glutamine and carbohydrates on digestive organs and pectoralis muscle mass in the duck. British Poultry Science, 50 (4), 436-442.

Christensen, V. L., Grimes, J. L., Donaldson, W. E,, Lerner, S., 2000. Correlation of body weight with hatchling blood glucose concentration and its relationship to embryonic survival. Poultry Science, 79, 1817-1822.

Coskun, I., Erener, G., Sahin, A., Karadavut, U., Altop, A., Okur, A.A., 2014. Impacts of In Ovo Feeding of DL-Methionine on Hatchability and Chick Weight. Turkish Journal of Agriculture - Food Science and Technology, 2(1), 47-50.

Erdogan, Y., Dodologlu, A., 2005. Importance of Pollen In Life of Honeybee (Apis meelifera L.) Colonies. Uludag Bee Journal, 5(2), 79-84.

Ferket, P. R., 2006. Incubation and in ovo nutrition affects neonatal development. 33rd Annual Carolina Poultry Nutrition Conference. 18-30. Newyork.

Foye, O. T., Ferket, P. R., Uni Z., 2007. The Effects of In Ovo Feeding Arginine, β-Hydroxy-β-Methyl- Butyrate, and Protein on Jejunal Digestive and Absorptive Activity in Embryonic and Neonatal Turkey Poults. Poultry Science, 86, 2343-2349.

Hame, M. J. Dickson, A. J., 1989. Influence of developmental stage on glycogenolysis and glycolysis in hepatocytesisolated from chick embryos and neonates. Biochemical Society transactions, 17, 1107-1108.

Ipek, A., Sahan, U., Yılmaz B., 2004. The effect of in ovo ascorbic acid and glucose injection in broiler breeder eggs on hatchability and chick weight. Arch Geflugelk, 68 (3), 132-135.

Keralapurath, M. M., Corzo, A., Pulikanti, R., Zhai, W., Peebles, E.D., 2010. Effects of in ovo injection of L-carnitine on hatchability and subsequent broiler performance and slaughter yield. Poultry Science, 89, 1497-1501.

McGruder, B. M., Zhai, W., Keralapurath, M. M., Gerard, P. D., Peebles, E.D., 2011. Effects of in ovo Injection of Theophylline and Electrolyte Solutions on Hatchability and Growth of Broilers from Day 0 to Day 10 Post-Hatch. International Journal of Poultry Science, 10 (12), 927-932.

Ohta, Y., Tsushima, N., Koide, K., Kidd, M. T., Ishibashi, T., 1999. Effect of Amino Acid Injection in Broiler Breeder Eggs on Embryonic Growth and Hatchability of Chicks. Poultry Science, 78, 1493-1498,

Ohta, Y., Kidd, M. T., 2001. Optimum site for in ovo amino acid injection in broiler breeder eggs. Poultry Science, 80, 1425-1429.

Salmanzadeh, M., Ebrahimnezhad, Y. H., Aghdam, S., Beheshti, R., 2012. The effects of in ovo injection of glucose and magnesium in broiler breeder eggs on hatching traits, performance, carcass characteristics and blood parameters of broiler chickens. Arch Geflügelk, 76 (4), 277-284.

Salmanzadeh, M., Ebrahimnezhad, Y. H., Aghdam, S., Lotfi, A., 2011. The effects of in ovo injection of L-threonine in broiler breeder eggs on characters of hatching and growth performance broiler chickens. European Journal of Experimental Biology, 1(4), 164-168.

Uni, Z., Ferket, P. R., Tako, E., Kedar, O., 2005. In Ovo Feeding Improves Energy Status of Late-Term Chicken Embryos. Poultry Science. 84:764-770.

Vieira, S.L., Moran, E. T., 1999. Effects of delayed placement and used litter on broiler yields. Journal of Applied Poultry Research, 8, 75-81.

Wang, J., Shenghe, L., Qifa, W., Baozhong, X., Heng, W., 2007. Journal of Medicinal Food, 10(2), 276-280.

Zhai, W., Gerard, P. D., Pulikanti, R., Peebles, E. D., 2011. Effects of in ovo injection of carbohydrates on embryonic metabolism, hatchability, and subsequent somatic characteristics of broiler hatchlings. Poultry Science, 90, 2134-2143.

Zhai, W., Neuman, S., Latour, M. A., Hester, P. Y., 2008. The Effect of In Ovo Injection of L-Carnitine on Hatchability of White Leghorns. Poultry Science, 87, 569-572.