Effects of In-ovo Injection of Different Nutrients on the Hatchability and growth Performance in Broilers

M. Eslami¹, M. Salarmoini²* and S. Tasharrofi³

¹²Graduate Student and Associate Professor, respectively, Faculty of Agriculture, Department of Animal Science, Shahid Bahonar University of Kerman, Kerman, Iran.
³Consultant of Agricultural Organization of Kerman, Iran.
* Corresponding author, E-mail address: salarmoini@uk.ac.ir

Abstract The aim of this study was to investigate the effects of in ovo injections of albumin, amino acid and dextrose into the amnion on the hatchability, growth performance and slaughter yield of Ross 308 broiler chicks. Fertile eggs (n=360) were assigned to 6 treatment groups (4 replicates of 15 eggs each) including: non-injected eggs (control), and eggs injected with 0.7 ml distilled water (sham), amino acids, albumin 20%, dextrose 20% and dextrose 10% . The injections were made on d 17.5 of the incubation period, and the hatch rate of fertilized eggs were recorded. Post-hatch performance of was determined weekly up to day 42. On d 42 of age, the weight of carcasses, thighs, wings, breast muscle, back and neck were determined. The results showed that in ovo injection of albumin increased body weight (P<0.05) on the first d compared to the control and shame groups. There were no significant effects of treatments on feed intake, feed conversion ratio, carcass characteristics and slaughter yields.

Keywords: amino acids, albumin, dextrose, in-ovo injection, broiler


Introduction

Under commercial industry practices and current standard feeding procedures, hatchlings are commonly held for 36 to 72 h from the time of actual hatch to placement on the farm (Decuypere et al., 2001). Under practical conditions, many birds do not have access to feed until 48 h after hatching. This has resulted in 7.8% decrease in body weight (BW) (Noy and Sklan, 1998). Under fasting conditions, hatchlings became more susceptible to pathogens (Dibner et al., 2008), their BW decreased (0.18 g/h) (Bigot et al., 2003 and Careghi et al., 2005), and experienced restricted developments in critical tissues and organs, such as the intestine (Geyra et al., 2001; Dibner and Richards 2004), immune system (Dibner et al., 2008) and pectoral muscle (Halevy et al., 2003; Moore et al., 2005). Several strategies have been proposed to improve performance during this initial phase, such as feeding at the hatchery (Dibner et al., 1998; Careghi et al., 2005), and in ovo feeding (Foye et al., 2006 and Tako et al., 2004) . In ovo injection technology is a practical means for safe introduction of nutrients into developing embryos, including amino acids (Ohta et al., 1999; Kadam et al., 2008), carbohydrates (Tako et al., 2004), vitamins (Gore and Qureshi, 1997), L-carnitine (Zhai et al., 2008; Keralapurath et al., 2010), and hormones (Henry and Burke, 1999; Kocamis et al., 2000) which may benefit posthatch growth and BW gain. Amnion is an efficient site for in ovo injection (Zhai et al., 2008; Keralapurath et al., 2010). Substances in the amnion enter the embryo through the mouth and can be absorbed through the intestine, respiratory tract, and lungs (Jochemsen and Jeurissen, 2002). Exogenous carbohydrates, as readily available energy sources, may help spare proteins and fatty acids that would normally be used for gluconeogenesis so that embryo growth may be optimized (Uni and Ferket, 2004; Foye et al., 2006; Bottje et al., 2010). Injection of a solution of carbohydrates and β-hydroxy-β-methylbutyrate (a leucine metabolite) into the amniotic fluid of broiler embryos 3 to 4 d before hatch replenished the glycogen stores during the prenatal period and increased BW and pectoral muscle-to-BW ratio (Uni et al., 2005). In ovo injection of a 1.0-mL volume of various combinations of carbohydrates at a concentration of 0.18 to 0.25 g/mL improved chick BW at hatch (Tako et al., 2004; Uni et al., 2005; Smirnov et al., 2006). Also, in ovo injection of a mixture of carbohydrates dissolved in saline [sucrose (a mo-
nosaccharide), maltose (a disaccharide), and dextrin (a polysaccharide) with or without β-hydroxy-β-methylbutyrate (HMB, a leucine metabolite) at d 17 or 17.5 of incubation improved embryonic intestinal development and increased total chick BW at hatch (Tako et al., 2004; Uni and Ferket, 2004; Uni et al., 2005; Smirnov et al., 2006). Ohta et al. (2001) suggested that higher BW of 7-day-old chicks following the injection of amino acids in to embryos would be related to the higher content of amino acids in the yolk or to better utilization of amino acids by the embryo. Halevy et al. (2000) observed lower BW and breast meat yield values at 41 d when broilers were fasted for 24 h after hatching in compare to those immediately fed, likely caused by a change in satellite cell activity, leading to subsequent changes in hyperplasia and an associated delay in muscle maturation.

The objective of this study was to evaluate the effects of in ovo feeding of several substances at d 17.5 of embryonic age on the hatch percentage, performance and carcass characteristics in broilers.

**Materials and methods**

**Incubation and injection**

Broiler hatching eggs (Ross 308), at 36 wk of age were obtained from a commercial flock. On d 17.5 of incubation, the eggs were individually weighed and only those that were not noticeably abnormal and were ±10% of the mean weight of all the eggs (58±2 g) were set randomly in each of 6 hatchery tray levels. Sixty eggs (4 replicates of 15 eggs) were assigned to each treatment group, and treatment groups were randomly represented at each tray level, with each tray level serving as a unit of replication. The injection site was disinfected with alcohol and then 0.7 ml of each solution was injected into the amnion, using a 23-gauge needle with depth of 25 mm from the broad end of the egg. The holes were then sealed using a commercial glue (Zhai et al., 2008).

The treatments were: without injection (control), and injection with distilled water (sham), amino acids¹, albumin² 20%, dextrose³ 20% or dextrose⁴ 10%. Control group

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>Starter (d 0 to 21)</th>
<th>Grower (d 22 to 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>53.63</td>
<td>63.56</td>
</tr>
<tr>
<td>Soybean meal (44%)</td>
<td>39</td>
<td>31.1</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.4</td>
<td>1.15</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.43</td>
<td>0.32</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Vitamin-mineral premix¹</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

**Calculated analysis**

<table>
<thead>
<tr>
<th>Component</th>
<th>Starter</th>
<th>Grower</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>21.41</td>
<td>18.81</td>
</tr>
<tr>
<td>AMEn kcal/kg</td>
<td>2980</td>
<td>3010</td>
</tr>
<tr>
<td>Lys, %</td>
<td>1.19</td>
<td>1.00</td>
</tr>
<tr>
<td>Met, %</td>
<td>0.48</td>
<td>0.38</td>
</tr>
<tr>
<td>Met + Cys, %</td>
<td>0.83</td>
<td>0.68</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.93</td>
<td>0.85</td>
</tr>
<tr>
<td>Av. P, %</td>
<td>0.41</td>
<td>0.33</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.18</td>
<td>0.14</td>
</tr>
<tr>
<td>Linoleic acid, %</td>
<td>3.14</td>
<td>2.52</td>
</tr>
</tbody>
</table>

¹Provided per kilogram of premix: vitamin A 3600000 IU; vitamin D3 80000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; pyridoxine, 1176 mg; thiamin, 700 mg; riboflavin, 2640 mg; pantothenic acid, 3920 mg; niacin, 11880 mg; biotin, 40 mg; choline, 200000 mg; folic acid, 400 mg; vitamin B12, 6 mg; antioxidant, 1000 mg; Se, 80 mg; Cu, 4000 mg; I, 396 mg; Mn, 39680 mg; Zn, 33880 mg.  
2-Fresenius Kabi Austria GmbH. (Austria), Each 1000 ml contained 200 g albumin, 2.31 g caprylate, 3.94 g N-acetyl-dl-tryptophanat and 2.8 g Na.  
3-Mashhad Samen Medical Co., Each 100 ml contained 20 g anhydrous dextrose.  
4-Each 100 ml contained 10 g anhydrous dextrose.
Effect of in-ovo Injection on Broiler traits

Bird housing

Hatched chicks of each treatment were randomly assigned to 4 pens per treatment (13 hatchlings/1.2×1.2 m² pen). Floor pens, equipped with manual self-feeder and drinker, were bedded with soft wood shavings. Chicks were raised under similar environmental conditions based on Ross 308 management recommendations (Aviagen, 2009) for 42 days, and had free access to feed and water. Diets were formulated according to NRC (1994) recommendations (Table 1).

Data collection

On the day of hatch (d 21 of incubation), hatchability was calculated and expressed as a percentage of fertilized eggs. The average body weight of birds in each pen was recorded on days 1, 7, 14, 21, 28, 35 and 42 of age. Feed intake and feed conversion ratio (FCR) were calculated weekly. On d 42, one bird per replicate was weighed and slaughtered. Carcass weight and major parts including the breast, thighs, wings, back and neck were measured and expressed as percentages of live BW.

Statistical analysis

Results were analyzed by ANOVA using the GLM procedure of SAS software (SAS Institute, 2008). Differences between treatments were compared by the Duncan’s multiple range test (P <0.05).

Results and discussion

In ovo injection did not have any negative effects on hatchability percentage (Table 2). In ovo injection of carbohydrate solutions and maltose, a multivitamin supplement, zinc-glycine, glutamine, or a mixture containing all these elements on d 18 or 18.5 of incubation did not affect rate of hatch (Zhai et al., 2011; Dos Santos et al., 2010). Pedroso et al (2006) reported that injection of GLU at d 16 did not influence rate of hatch but they observed lower hatchability when injecting glucose in the amniotic fluid. Ohta et al. (1999) observed that injection of amino acids at 0 d of incubation reduced hatchability. The results showed that in ovo injection of albumin increased d 1 BW compared to the control and sham groups. There was not any significant difference between treatments at other ages (Table 3). Rapid growth coupled with a high energy requirement, especially during late embryogenesis, may make in ovo feeding of supplemental carbohydrates beneficial to embryos. Muscles exclusively use glycogen derived glucose for a rapid and strong contraction that is essential to shell perforation and chick emergence (Moran, 2007). The inoculation of carbohydrates and protein, β-hydroxy-β-methylbutyrate (HMB), and carbohydrate increased hatching weight and BW of 7-d-old chicks (Foye et al., 2006). Chicken eggs are rich in protein and lipids but are poor in carbohydrates (Burley and Vadehra, 1989). For the homeostatic control of blood glucose levels. The embryos rely on gluconeogenesis to metabolize glycerol and amino acid substrates liberated by lipolysis and proteolysis, respectively (Klasing, 1998). Subsequently, increased gluconeogenic degradation of proteins toward the time of hatch may limit embryonic growth. Because the late-term embryo orally consumes the amniotic fluid (comprised primarily of water and albumen protein) prior to pipping, in ovo feeding of dextrose, amino acids or albumin may help to overcome any nutrient deficiency that may limit embryonic growth. Thus, it was hypothesized that administration of carbohydrates to the amnion may improve the energy level of the broiler embryo and reduce internal energy consumption (proteins and lipids) during pipping, thereby increasing chick BW (Zhai et al., 2011). Digested proteins provide free amino acids, the possible substrates for hepatic gluconeogenesis. The uptake of glucose and amino acids by skeletal muscle is mediated by the action of insulin (Xu et al., 1998). Studies have shown that dietary amino acids are important signaling mediators in pancreatic β-cell insulin secretion in vitro and release of insulin-like growth factors in vivo (Xu et al., 1998). Partic-

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Hatchability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-injected (control)</td>
<td>91.66</td>
</tr>
<tr>
<td>Distilled water (sham)</td>
<td>90.00</td>
</tr>
<tr>
<td>Amino acids</td>
<td>93.33</td>
</tr>
<tr>
<td>Albumin</td>
<td>93.33</td>
</tr>
<tr>
<td>Dextrose 20%</td>
<td>91.66</td>
</tr>
<tr>
<td>Dextrose 10%</td>
<td>88.33</td>
</tr>
<tr>
<td>SEM</td>
<td>3.621</td>
</tr>
<tr>
<td>P-value</td>
<td>0.912</td>
</tr>
</tbody>
</table>

Table 2. Effect of in-ovo injection of nutrients on egg hatchability
ularly, leucine has been shown to be an insulin secreta-
gogue and to potentiate glucose-stimulated insulin se-
cretion in pancreatic β-cells (Tsuruzoe et al., 1998). In-
sulin, an inhibitor of hepatic gluconeogenesis (Pocai et
al., 2005) may have metabolically shifted the use of di-
etary protein (in ovo feeding of protein) away from glu-
coneogenesis (Foye et al., 2006). Conversely, these die-
tary proteins (in ovo feeding of protein) may have be-
come more available to provide the building blocks for
muscle protein. These amino acids and peptides would
have been absorbed by the muscles due to the action of
insulin and incorporated into protein (Foye et al., 2006).
Therefore, albumin injection may increase hatchability
and body weight on the first day of hatch in comparison
to other treatments.

Leitão et al. (2006) did not observe any differences in
BW gain between 0 and 10 d in chicks supplemented
with glucose at 16 d of incubation. Lopes et al. (2006)
jected glutamine to 16 d of incubation. Lopes et al. (2006)
did not observe any differences in feed intake or feed conversion ratio
of chicks inoculated with glucose at 16 d of incubation
which are in agreement with the present study. In ovo
feeding did not have any significant effects on carcass
traits (Table 5). Previously published data also showed
that in ovo injection of several nutrients at 18 d of incu-
bation did not influence carcass traits (Dos Sontos et al.,
2010; Keralapurath et al., 2010; Doley et al., 2011).
Foye et al. (2006) observed higher breast weights at
 hatch in turkey poult injected with a protein solution at
23 d of incubation; however, in the present work, breast
weight at d 14 of growing period was not affected by in
ovo injection of several nutrients. Injection of carbohy-
drate solution in 18-d-old broiler embryos promoted
higher breast meat yields at hatching and at 7 d of age
(Uni and Ferket, 2004). Uni et al. (2005) observed
higher breast weight at hatch, 10 and 25 d of age in
chicks injected with carbohydrates and hydroxy-
methylbutyrate at 18 d of incubation which are different
from the results of the present experiment (injection of
dextrose and protein).

### Table 3. Effect of in ovo feeding of nutrients on live weight (g) in broilers from 1 to 42 days of age

<table>
<thead>
<tr>
<th>Treatments</th>
<th>d 1</th>
<th>d 7</th>
<th>d 14</th>
<th>d 21</th>
<th>d 28</th>
<th>d 35</th>
<th>d 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-injected (control)</td>
<td>38.06b</td>
<td>105.31</td>
<td>280.45</td>
<td>601.25</td>
<td>1018.86</td>
<td>1565.42</td>
<td>2128.27</td>
</tr>
<tr>
<td>Distilled water (sham)</td>
<td>37.97b</td>
<td>109.41</td>
<td>295.59</td>
<td>639.58</td>
<td>1090.83</td>
<td>1661.91</td>
<td>2223.60</td>
</tr>
<tr>
<td>Amino acid</td>
<td>39.15a,b</td>
<td>106.56</td>
<td>283.07</td>
<td>628.69</td>
<td>1063.18</td>
<td>1653.26</td>
<td>2291.70</td>
</tr>
<tr>
<td>Albumin</td>
<td>40.35a</td>
<td>110.72</td>
<td>293.52</td>
<td>603.63</td>
<td>1075.02</td>
<td>1628.56</td>
<td>2186.53</td>
</tr>
<tr>
<td>Dextrose 20%</td>
<td>39.15a,b</td>
<td>105.10</td>
<td>281.36</td>
<td>599.50</td>
<td>1029.55</td>
<td>1567.15</td>
<td>2029.55</td>
</tr>
<tr>
<td>Dextrose 10%</td>
<td>38.65b</td>
<td>105.31</td>
<td>272.50</td>
<td>626.74</td>
<td>1071.25</td>
<td>1643.84</td>
<td>2187.74</td>
</tr>
<tr>
<td>SEM</td>
<td>0.475</td>
<td>2.149</td>
<td>8.179</td>
<td>18.466</td>
<td>33.539</td>
<td>33.751</td>
<td>55.280</td>
</tr>
<tr>
<td>P-value</td>
<td>0.020</td>
<td>0.316</td>
<td>0.382</td>
<td>0.523</td>
<td>0.633</td>
<td>0.203</td>
<td>0.213</td>
</tr>
</tbody>
</table>

a,b Values within a column with different superscripts differ significantly (P < 0.05).

brows did not influence feed intake and feed conversion
(Dos Sontos et al., 2010; Keralapurath et al., 2010;
Doley et al., 2011). Pedroso et al. (2006) did not observe
any differences in feed intake or feed conversion ratio
of chicks inoculated with glucose at 16 d of incubation
which are in agreement with the present study. In ovo
feeding did not have any significant effects on carcass
traits (Table 5). Previously published data also showed
that in ovo injection of several nutrients at 18 d of incu-
bation did not influence carcass traits (Dos Sontos et al.,
2010; Keralapurath et al., 2010; Doley et al., 2011).
Foye et al. (2006) observed higher breast weights at
 hatch in turkey poult injected with a protein solution at
23 d of incubation; however, in the present work, breast
weight at d 14 of growing period was not affected by in
ovo injection of several nutrients. Injection of carbohy-
drate solution in 18-d-old broiler embryos promoted
higher breast meat yields at hatching and at 7 d of age
(Uni and Ferket, 2004). Uni et al. (2005) observed
higher breast weight at hatch, 10 and 25 d of age in
chicks injected with carbohydrates and hydroxy-
methylbutyrate at 18 d of incubation which are different
from the results of the present experiment (injection of
dextrose and protein).

### Conclusions

Injection of different nutrients into fertile eggs had no
detrimental effects on the hatch percentage, but albumin

### Table 4. Effect of in ovo feeding of nutrients on feed intake (g) and feed conversion ratio

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feed intake</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-21 days</td>
<td>22-42 days</td>
</tr>
<tr>
<td>Non-injected (control)</td>
<td>864.2</td>
<td>3317.0</td>
</tr>
<tr>
<td>Distilled water (sham)</td>
<td>906.7</td>
<td>3517.1</td>
</tr>
<tr>
<td>Amino acid</td>
<td>879.8</td>
<td>3266.5</td>
</tr>
<tr>
<td>Albumin</td>
<td>870.2</td>
<td>3377.7</td>
</tr>
<tr>
<td>Dextrose 20%</td>
<td>868.4</td>
<td>3227.2</td>
</tr>
<tr>
<td>Dextrose 10%</td>
<td>852.8</td>
<td>3418.2</td>
</tr>
<tr>
<td>SEM</td>
<td>24.83</td>
<td>125.663</td>
</tr>
<tr>
<td>P-value</td>
<td>0.736</td>
<td>0.659</td>
</tr>
</tbody>
</table>
injection seemed to improve chicks birth weight (P<0.05) although subsequent growth performance and slaughter yield at post-hatch period were not affected.

**References**


Effect of in-ovo Injection on Broiler traits

چکیده
در این تحقیق اثرات تزریق آلبومن، اسیدهای آمینه و دکستروز بر قابلیت جوجه کشی، عملکرد رشد و بازدهی لاشهه جوجه های گوشتی سویه را 360 تخم مرغ برشی قرار گرفت. در این آزمایش از 360 عدد تخم مرغ بارور برای 6 تیمار زیر استفاده شد: بدون تزریق (شاهد) و تزریق مواد غذایی به آمبیون شامل 0/7 میلی لیتر آب مقطر، اسیدهای آلبومن، آلبومن 1/2، دکستروز 1/2 و دکستروز 1/2. به هر تیمار 4 تکرار حاوی 0/1 تخم مرغ اختصاص داده شد. تزریق در روز 1/07، جوجه کشی انجام گردید و میزان قابلیت جوجه کشی و بدابا آیپتی قابلیت جوجه کشی به طور هفته‌ای و تا سه‌روز مورد بررسی قرار گرفت. در روزانه یک جوجه از هر تکرار انتخاب و قسمت‌های مختلف لاشه توزین گردید. براساس نتایج، در یک روزگر تزریق آلبومن، نسبت به تیمار برشی و آب مقطر سبب افزایش وزن گردید. تیمارها نتایج معنی‌داری بر مصرف خوراک، ضریب تبدیل و خصوصیات لاشه نداشته‌اند.

sairarmoini@uk.ac.ir

نویسنده: م. اسلامی، م. سالارمعینی و ش. تشرفی

Title:
بررسی تأثیر تزریق مواد مغذی بر قابلیت جوجه کشی و عملکرد جوجه‌های گوشتی

Author:
م. اسلامی، م. سالارمعینی و ش. تشرفی

E-mail:
sairarmoini@uk.ac.ir