

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

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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 day of hatch 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

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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 (Decuyper 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-d-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 $\pm 10\%$ 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 gr-

Table 1. Composition of starter and grower diets

Ingredient (%)	Starter (d 0 to 21)	Grower (d 22 to 42)
Corn	53.63	63.56
Soybean meal (44%)	39	31.1
Soybean oil	3.6	2.0
Calcium carbonate	1.3	1.3
Dicalcium phosphate	1.4	1.15
NaCl	0.43	0.32
DL-Methionine	0.14	0.07
Vitamin-mineral premix ¹	0.50	0.50
Calculated analysis		
CP, %	21.41	18.81
AME _n , kcal/kg	2980	3010
Lys, %	1.19	1.00
Met, %	0.48	0.38
Met + Cys, %	0.83	0.68
Ca, %	0.93	0.85
Av. P, %	0.41	0.33
Na, %	0.18	0.14
Linoleic acid, %	3.14	2.52

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; Fe, 2000 mg; Mn, 39680 mg; Zn, 33880 mg.

1-B. Braun Co. (Germany), Each 1000 ml amino acids contained 5.10 g isoleucine, 8.90 g leucine, 5.60 g lysine, 3.80 g methionine, 5.10 g phenylalanine, 4.10 g threonine, 1.80 g tryptophan, 4.80 g valine, 9.20 g arginine, 5.20 g histidine, 13.70 g alanine, 7.90 g glycine, 8.90 g proline, 2.40 g serine, 0.30 g tyrosine and 1.3 g aspartic acid, 3.27 g asparagine, 0.50 g cysteine, 4.60 g glutamic acid and 2.51 g Ornithine. Total amino acid: 100 g/l. Total N: 16

g/l. Total energy: 400 kcal/l.

2-Fresenius Kabi Austria GmbH. (Austria), Each 1000 ml contained 200 g albumin, 2.31 g caprylate, 3.94 g N-acetyl-dl-tryptophan 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.

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oup were taken out of the incubator for 20 min to equalize the conditions for all treatment groups.

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 ob-

served 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 shame 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 2. Effect of *in-ovo* injection of nutrients on egg hatchability

Treatments	Hatchability (%)
Non-injected (control)	91.66
Distilled water (sham)	90.00
Amino acids	93.33
Albumin	93.33
Dextrose 20%	91.66
Dextrose 10%	88.33
SEM	3.621
P-value	0.912

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

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

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

ularly, leucine has been shown to be an insulin secretagogue and to potentiate glucose-stimulated insulin secretion in pancreatic β -cells (Tsuruzoe et al., 1998). Insulin, an inhibitor of hepatic gluconeogenesis (Pocai et al., 2005) may have metabolically shifted the use of dietary protein (*in ovo* feeding of protein) away from gluconeogenesis (Foye et al., 2006). Conversely, these dietary proteins (*in ovo* feeding of protein) may have become 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) injected glutamine to 16-d-old embryos and did not find BW gain differences in 10- to 21-d-old broilers. In turkeys, addition of 10 IU of vitamin E did not influence the broiler BW at hatch and 35 d of age (Gore and Qureshi, 1997). Feed intake and feed conversion ratio (Tables 4) were not affected by *in ovo* inoculation of nutritional solutions at d 17.5 of incubation. *In ovo* inoculation of several nutrients (maltose, a multi-vitamin supplement, zinc-glycine, glutamine and a mixture containing all these elements and L-carnitin) to 18-d-old em-

bryos 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 incubation 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 poultts 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 carbohydrate 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 hydroxymethylbutyrate 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

Treatments	Feed intake			FCR		
	1-21 days	22-42 days	1-42 days	1-21 days	22-42 days	1-42 days
Non-injected (control)	864.2	3317.0	4181.2	1.47	2.20	2.01
Distilled water (sham)	906.7	3517.1	4423.8	1.54	2.29	2.05
Amino acid	879.8	3266.5	4146.3	1.51	2.02	1.86
Albumin	870.2	3377.7	4235.6	1.56	2.14	1.96
Dextrose 20%	868.4	3227.2	4078.4	1.57	2.16	1.98
Dextrose 10%	852.8	3418.2	4271.1	1.50	2.20	1.99
SEM	24.83	125.663	126.975	0.030	0.096	0.069
P-value	0.736	0.659	0.554	0.551	0.535	0.589

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Table 5. Effect of *in ovo* feeding of nutrients on carcass traits at d 42 of age (% live BW)

Treatments	Carcass	Breast	Thighs	Wings	Back	Neck
Non-injected (control)	64.1	38.1	26.8	9.0	22.6	3.2
Distilled water (sham)	65.1	38.0	28.8	8.9	22.1	3.0
Amino acid	61.6	39.7	27.7	9.2	21.7	3.9
Albumin	64.8	38.3	28.1	8.9	21.4	3.0
Dextrose 20%	64.5	38.5	28.7	9.0	20.3	3.2
Dextrose 10%	64.1	37.4	27.7	9.3	22.4	3.1
SEM	1.440	1.821	0.920	0.256	0.819	0.219
P-value	0.601	0.963	0.793	0.825	0.437	0.921

injection seemed to improve chicks birth weight ($P < 0.05$) although subsequent growth performance and slaughter yield at post-hatch period were not affected.

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چکیده در این تحقیق اثرات تزریق آلبومین، اسیدهای آمینه و دکستروز بر قابلیت جوجه کشی، عملکرد رشد و بازدهی لاشه جوجه های گوشتی سویه راس ۳۰۸ مورد بررسی قرار گرفت. در این آزمایش از ۳۶۰ عدد تخم مرغ بارور برای ۶ تیمار زیر استفاده شد: بدون تزریق (شاهد) و تزریق مواد مغذی زیر به آمینون شامل ۰/۷ میلی لیتر آب مقطر، اسیدهای آمینه، آلبومین ۲۰٪، دکستروز ۲۰٪ و دکستروز ۱۰٪. به هر تیمار ۴ تکرار حاوی ۱۵ تخم مرغ اختصاص داده شد. تزریق در روز ۱۷/۵ جوجه کشی انجام گردید و میزان قابلیت جوجه کشی و بدنبال آن میزان عملکرد آنها به طور هفتگی و تا سن ۴۲ روزگی مورد بررسی قرار گرفت. در پایان دوره یک جوجه از هر تکرار انتخاب و قسمت های مختلف لاشه توزین گردید. براساس نتایج، در یک روزگی تزریق آلبومین، نسبت به تیمار شاهد و آب مقطر سبب افزایش وزن گردید. تیمارها تأثیر معنی داری بر مصرف خوراک، ضریب تبدیل و خصوصیات لاشه نداشتند.