Effect of In Ovo Feeding Egg White Protein, β-Hydroxy-β-Methylbutyrate, and Carbohydrates on Glycogen Status and Neonatal Growth of Turkeys

O. T. Foye,* Z. Uni,† and P. R. Ferket*

*Department of Poultry Science, North Carolina State University, Raleigh 27695-7608; and †Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Rehovot, 76100 Israel

ABSTRACT In ovo feeding (IOF), injecting dietary components into the amnion about 1 d prior to internal pipping, may enhance growth by altering glycogen status. This hypothesis was evaluated with 5 IOF solutions containing protein, β-hydroxy-β-methylbutyrate (HMB), and carbohydrate. Four IOF treatments were arranged as a factorial of 2 levels of egg white protein (EWP; 0 and 18%) and 2 levels of HMB (0 and 0.1%). An IOF solution of carbohydrates (S; 20% dextrin and 3% maltose) was evaluated for contrast purposes. At 23 d of incubation, 1.5 mL of IOF solution was injected into the amnion of 100 eggs per treatment. At hatch, feed and water were provided ad libitum. At hatch and 3 and 7 d of age, BW were determined, and 10 poults per treatment were sampled to determine liver (LG) and pectoralis muscle (PC) glycogen content. Poults on IOF treatments A (18% EWP), B (18% EWP + HMB), and D (HMB) weighed 6.0, 2.7, and 3.3% more than the controls at hatch, respectively ($P < 0.05$) with an EWP × HMB interaction ($P < 0.05$) sustained to 3 and 7 d only in treatment D ($P < 0.005$). At hatch, A and D poults had greater percentages of PC ($P < 0.05$) than controls, and the percentage of PC in treatment D was sustained until 7 d. Total LG was enhanced by A and B at 7 d ($P < 0.05$) over the controls, whereas total PC glycogen was enhanced at 7 d by IOF treatment D ($P < 0.05$). The IOF A and S poults had greater BW than the controls at hatch only ($P < 0.05$). The IOF treatment A had greater LG at hatch ($P < 0.05$), but by 7 d, A and S had greater LG than controls ($P < 0.05$). Poults fed S in ovo had enhanced total PC glycogen over controls, whereas poults on treatment A had less total PC glycogen than controls ($P < 0.05$). The results of this experiment demonstrate that IOF of A or S poults may enhance hatch BW and glycogen status of poults during the neonatal period by inclusion of HMB.

Key words: in ovo feeding, liver glycogen, pectoralis muscle glycogen, body weight, turkey

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INTRODUCTION

The developing chick embryo must rely upon the nutrients provided by the egg independently of maternal influence. Nutrient transfer from the mother to the embryo is completed before the egg is laid, so the egg contains all of the nutrients needed for the growth and development of the embryo. The in ovo (**IO**) nutriture of the chick embryo consists mainly of yolk fat with traces of carbohydrates (Starck and Rickelefs, 1998). However, glucose is the primary source of carbohydrate energy needed for development, growth, and maintenance and is an important component of the cellular membranes, glycoproteins, and glycolipids. Posthatch, birds switch from a fat-based energy nutriture to more carbohydrate-based as the neonatal chick adapts to a high-carbohydrate corn diet.

Glycogen reserves in the avian embryo provide the critical energy needed for emergence from the egg during the last quarter of incubation. In turkeys, extensive embryonic mortality occurs toward the end of the incubation period when hatching-related events occur, such as pipping of the egg membrane and shell, beginning of pulmonary respiration, and the actual egg emergence (Christensen and Donaldson, 1992; Christensen et al., 2000, 2001). Glycogen reserves in the chick embryo are significantly depleted during the perihatch period to meet the high energy demand during the process of emergence (Freeman, 1965, 1969; Freeman and Manning, 1971). Liver and muscle glycogen reserves are depleted due to carbohydrate use for muscular activity during the hatching process (George and Iype, 1963; Bakhuis, 1974; John et al., 1987, 1988) and for posthatch growth, activity, and maintenance (Warriss et al., 1988).

A novel method of supplementing the IO nutriture of oviparous species, described as in ovo feeding (**IOF**) within the US Patent (6,592,878) of Uni and Ferket (2003), involves the administration of exogenous nutrients into the amnion of the developing embryo of chickens and turkeys at about 17 and 23 d of incubation, respectively. Because the late-term embryo orally consumes the amniotic fluid (comprised primarily of water and albumen

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Corresponding author: Peter_Ferket@ncsu.edu

protein) prior to pipping of the air cell, this IOF technology is a means of presenting exogenous nutrients to the enteric tissues for absorption and use for growth and stored energy as glycogen. Therefore, IOF—a supplemental source of protein and carbohydrate—may help overcome the constraint of limited egg nutriture. Consequently, the enhanced glycogen stores may provide supplemental energy needed for better neonatal survival, fuel more rapid growth, and spare body protein (muscle) reserves posthatch. Hatchlings with enhanced glycogen reserves have improved BW, decreased mortality, and improved performance (Moran, 1988, 1989, 1990).

Uni and Ferket (2003) demonstrated that turkey poults IO fed β-hydroxy-β-methylbutyrate (**HMB**), a leucine metabolite (0, 0.1, 1.0, and 10 μg) had approximately a 40% increase in liver glycogen (**LG**) over the injected and noninjected controls, with a quadratic response as the level of HMB injected IO increased. Moreover, hatchability rates were positively correlated with LG content of turkey and chick embryos before hatch (Uni and Ferket, 2003). Additional experiments revealed that IOF carbohydrates and protein (Uni and Ferket, 2004), carbohydrates, HMB, or both carbohydrates and HMB (Uni et al., 2005) increased broiler hatching BW, relative pectoralis breast muscle, and improved LG reserves over the controls.

Thus we hypothesize that IOF of HMB, protein, or carbohydrates may enhance body glycogen reserves and growth in the avian embryo and neonate. Our objectives were to evaluate the effects of IOF solutions containing egg white protein (**EWP**; predominant protein source within the egg), HMB, or both EWP and HMB on somatic growth and total LG and muscle glycogen status of the neonatal turkey, in contrast to an IOF solution of a highly digestible carbohydrate source (dextrin and maltose) not to exceed osmolality limits.

MATERIALS AND METHODS

Incubation and IOF

Hybrid turkey eggs were obtained at 19 d of incubation from a commercial hatchery (Prestage Farms, Clinton, NC) and incubated according to standard hatchery practices (37.5°C). At 21 d of incubation, 500 eggs were individually weighed and distributed among four 5-g weight categories ranging from 65 to 70, 71 to 75, 76 to 80, and 81 to 85 g. These eggs were then evenly distributed among 5 treatment groups of 100 eggs each, such that the weight distribution profile among all 5 treatment groups was identical. At 23 d of incubation, each egg was candled to identify the location of the amnion. A hole was then punched using a 23-gauge needle and 1.5 mL of IOF solution injected into the amnion using a 23-gauge needle to a depth of about 15 mm. The injection hole area was disinfected with an ethyl alcohol-laden swab, sealed with cellophane tape, and transferred to hatching baskets. The IOF solutions were prepared as asceptically as possible such that the IOF treatment solutions contained the following: A, 18% EWP (Sigma Chemical Co., St. Louis, MO) in 0.9% saline; B, 18% EWP + 0.1% HMB in 0.9% saline; D, 0.1% HMB (calcium salt, Metabolic Technologies, Inc., Ames, IA) in 0.9% saline; and S, 20% dextrin (Sigma Chemical Co.) + 3% maltose (Sigma Chemical Co.) in 0.9% saline. The controls (treatment C) were not injected but they were subjected to the same handling procedures as the IOF treatment groups.

Animal Husbandry

Upon hatching, each poult was identified by neck tag number and BW recorded at hatch and 7 d posthatch. Hatchability rate of viable eggs was >95% and did not differ significantly among treatment groups. All the birds were housed together in one room of a total confinement building with supplemental heat from propane-fired heaters to maintain about 27°C. Each floor pen was bedded with soft pine wood shavings and equipped with automatic nipple drinkers, a manual self-feeder, and supplemental incandescent heat lamp to maintain a brooding temperature of approximately 40°C. The poults were provided ad libitum access to a typical turkey starter diet (2,935 kcal/kg, 27.5% protein, and 5.6% fat) that met or exceeded National Research Council (1994) nutrient requirements for turkeys. All experimental protocols were approved by the Institutional Animal Care and Use Committee at North Carolina State University.

Tissue Sampling and Glycogen Analysis

At hatch and 7 d posthatch, 10 birds per treatment were euthanized by cervical dislocation and within 2 min the whole liver and pectoralis muscle was dissected and placed on ice before freezing for subsequent glycogen analysis. Frozen liver or muscle samples were then thawed in groups such that all sample days and treatments were equally represented so as to account for errors associated with glycogen analysis. The pectoralis muscle and liver samples were then homogenized in 8% perchloric acid (1 g/4 mL), and glycogen content was determined using modified methods described by Dreiling et al. (1987). After homogenization, the samples were centrifuged at 14,000 rpm at 4°C for 30 min. One milliliter of the supernatant was transferred to a clean polypropylene tube, and 2.0 mL of petroleum ether was added to each sample and vortexed. The samples were centrifuged at 2,000 rpm at 4°C for 15 min. Subsequently, a 10-μL aliquot of sample (from the bottom layer) was added to a disposable cuvette, along with 0.4 mL of 8% perchloric acid and 2.6 mL of iodine color reagent made of 1.3 mL of solution A (0.26 g of iodine + 2.6 g of potassium iodide dissolved in 10 mL of distilled water) in 100 mL of 67.8% saturated calcium chloride (anhydrous) solution. All samples were read at a wavelength of 460 nm. The amount of glycogen present in a 10-μL sample is determined by preparation of a known glycogen standard curve.

Statistical Analysis

All data were analyzed statistically using GLM procedures for ANOVA (SAS Institute, 1996). Each bird served

Table 1. The effects of in ovo feeding (IOF) β-hydroxy-β-methylbutyrate (HMB) and egg white protein (EWP) on the BW of turkeys at hatch, 3 and 7 d posthatch (g)

	Hatch		Day 3		Day 7	
IOF treatment[1]	Mean	n	Mean	n	Mean	n
A (protein)	56.02[a]	55	73.23[a]	45	131.91[c]	45
B (protein + HMB)	54.09[b]	45	76.63[a]	35	140.93[ab]	35
C (control)	52.64[c]	76	73.64[a]	67	135.47[bc]	66
D (HMB)	54.46[b]	53	75.84[a]	45	145.75[a]	43
			P-value			
Source of variation						
Protein	0.0008		0.882		0.157	
HMB	0.905		0.0324		0.001	
Protein × HMB	<0.0001		0.642		0.831	
SEM (df)	0.217 (227)		0.630 (186)		1.44 (185)	

[a–c]Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]Treatment A IOF solution contained 18% EWP in 0.9% NaCl saline. Treatment B IOF solution contained 18% EWP + 0.1% HMB in 0.9% NaCl saline. Treatment C is the noninjected control. Treatment D IOF solution contained 0.1% HMB in 0.9% NaCl saline.

as an experimental unit for statistical analysis. Because highly significant age effects were observed, the treatment effects were evaluated by neonatal age (i.e., hatch, 3 and 7 d of age). Data from IO treatments A, B, control, and D were analyzed as a 2 × 2 factorial arrangement, with 2 levels of EWP (0 and 18%) and 2 levels of HMB (0 and 0.1%). Variables having different *F*-test were compared using the least square means function in the SAS software (1996), and the treatment effects were considered significant at $P < 0.05$. An additional analysis was conducted to contrast the effects of dietary carbohydrates vs. dietary protein on liver and muscle glycogen content. These data were analyzed as a 1-way ANOVA with treatments A (18% EWP in 0.9% saline), control (noninjected control), and S (20% dextrin and 3% maltose in 0.9% saline). When ANOVA tests were significant ($P < 0.05$), the treatments were separated by least squares means (*t*-test). All experiments were conducted with an equal frequency of variables within each treatment.

RESULTS

Main Effects of EWP and HMB Levels of IOF

At hatch, the BW of all IO-fed poults were significantly greater than the controls (Table 1), and there was a highly significant EWP × HMB effect observed. Poults IO fed with 18% EWP, a combination of 18% EWP + HMB, or 0.1% HMB had BW that were 6.0, 2.7, and 3.3% greater than the controls, respectively (Table 1). This effect was lost by 3 d posthatch; however, a significant main effect of HMB appeared and persisted until 7 d. Whereas the poults fed IO with EWP + HMB and HMB alone were larger than the controls, their values were statistically similar. At 7 d posthatch, only the poults fed HMB IO had significantly greater BW in comparison with the controls (Table 1).

Table 2. The effects of in ovo feeding (IOF) β-hydroxy-β-methylbutyrate (HMB) and egg white protein (EWP) on relative pectoralis muscle and liver mass of turkeys at hatch and 7 d posthatch[1]

	Pectoralis muscle		Liver	
IOF treatment[2]	Hatch	Day 7	Hatch	Day 7
	% of BW			
A (protein)	3.30[a]	9.20[a]	2.60[a]	3.00[a]
B (protein + HMB)	2.80[b]	9.80[a]	2.40[a]	3.20[a]
C (control)	2.70[b]	9.30[a]	2.40[a]	2.90[a]
D (HMB)	3.10[ab]	10.00[a]	2.40[a]	3.00[a]
	P-value			
Source of variation				
Protein	0.241	0.588	0.255	0.262
HMB	0.713	0.050	0.172	0.090
Protein × HMB	0.002	0.873	0.334	0.550
SEM (df)	0.06 (36)	0.167 (36)	0.083 (36)	0.088 (36)

[a,b]Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]All data represent the mean value ± SE of 10 sample birds per treatment.

[2]Treatment A IOF solution contained 18% EWP in 0.9% NaCl saline. Treatment B IOF solution contained 18% EWP + 0.1% HMB in 0.9% NaCl saline. Treatment C is the noninjected control. Treatment D IOF solution contained 0.1% HMB in 0.9% NaCl saline.

Table 3. The effects of in ovo feeding (IOF) β-hydroxy-β-methylbutyrate (HMB) and egg white protein (EWP) on liver glycogen concentration and total liver glycogen of turkeys at hatch and 7 d posthatch[1]

	Glycogen concentration (mg/g)		Total glycogen (mg)	
IOF treatment[2]	Hatch	Day 7	Hatch	Day 7
A (protein)	36.18[a]	140.54[a]	52.50[a]	590.53[a]
B (protein + HMB)	22.00[bc]	154.91[a]	28.30[b]	682.93[a]
C (control)	16.44[c]	84.68[b]	20.30[b]	378.64[b]
D (HMB)	25.69[ab]	66.83[c]	32.50[b]	296.90[b]
	P-value			
Source of variation				
Protein	0.123	0.004	0.061	0.009
HMB	0.631	0.940	0.414	0.961
Protein × HMB	0.027	0.492	0.017	0.424
SEM (df)	2.54 (36)	11.60 (36)	3.62 (36)	53.83 (36)

[a–c]Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]All data represent the mean value ± SE of 10 sample birds per treatment.

[2]Treatment A IOF solution contained 18% EWP in 0.9% NaCl saline. Treatment B IOF solution contained 18% EWP + 0.1% HMB in 0.9% NaCl saline. Treatment C is the noninjected control. Treatment D IOF solution contained 0.1% HMB in 0.9% NaCl saline.

Poults IO fed with 18% EWP had significantly greater pectoralis muscle size relative to BW at hatch in comparison with the controls (Table 2). At 3 d posthatch, there were marginal differences in the relative pectoralis mass between the IO-fed poults and controls but a significant positive effect of HMB on relative pectoralis mass (data not shown). There was no significant difference in the relative pectoralis mass between poults IOF EWP or 20% dextrin + 3% maltose (**S**) at hatch or 7d posthatch (data not shown). Additionally, the relative liver mass of poults IOF 18% EWP or HMB or both and the controls were similar at hatch and 7 d posthatch (Table 2).

Poults IO fed EWP had the heaviest BW and the greatest relative pectoralis muscle mass at hatch (Table 1 and Table 2, respectively). Evidently, neither BW nor pectoralis muscle yield was enhanced further at hatch by the addition of HMB to the IOF solution. Poults IO fed EWP + HMB or HMB alone had similar BW and pectoralis muscle yields, revealing a significant EWP × HMB interaction effect. But this significant EWP × HMB effect was no longer observed 3 and 7 d posthatch. In contrast, IOF of HMB alone had the greatest effect on BW and relative pectoralis mass 3 and 7 d posthatch (Table 1 and Table 2, respectively).

Total LG and LG concentrations were enhanced significantly at hatch over the controls by IOF of EWP with a significant EWP × HMB interaction (Table 3). The IOF of HMB significantly enhanced the concentration of LG over the controls at hatch. There was a highly significant EWP effect, such that poults IO fed EWP alone and EWP + HMB had significantly enhanced total LG reserves and concentrations at 1 wk posthatch, in comparison to the controls.

Total pectoral muscle glycogen (**PC**) was significantly enhanced at hatch and 7 d posthatch by IOF of HMB alone over the controls, with HMB and EWP independently having significant main effects (Table 4). The inclusion of EWP in the IO nutrient solution depressed total

Table 4. The effects of in ovo feeding (IOF) β-hydroxy-β-methylbutyrate (HMB) and egg white protein (EWP) on pectoralis muscle glycogen concentration and total pectoralis muscle glycogen of turkeys at hatch and 7 d posthatch[1]

	Glycogen concentration (mg/g)		Total glycogen (mg)	
IOF treatment[2]	Hatch	Day 7	Hatch	Day 7
A (protein)	9.18[c]	37.61[b]	16.88[d]	469.59[d]
B (protein + HMB)	24.05[b]	41.14[b]	36.49[c]	545.92[c]
C (control)	40.74[a]	52.30[a]	56.12[b]	693.50[b]
D (HMB)	42.96[a]	55.72[a]	70.32[a]	790.76[a]
	P-value			
Source of variation				
Protein	<0.0001	<0.0001	<0.0001	<0.001
HMB	0.0002	0.072	<0.0001	0.0016
Protein × HMB	0.0046	0.978	0.483	0.691
SEM (df)	1.05 (36)	0.937 (36)	1.92 (36)	12.71 (36)

[a–d]Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]All data represent the mean value ± SE of 10 sample birds per treatment.

[2]Treatment A IOF solution contained 18% EWP in 0.9% NaCl saline. Treatment B IOF solution contained 18% EWP + 0.1% HMB in 0.9% NaCl saline. Treatment C is the noninjected control. Treatment D IOF solution contained 0.1% HMB in 0.9% NaCl saline.

Table 5. The effects of in ovo feeding (IOF) β-hydroxy-β-methylbutyrate (HMB) and egg white protein (EWP) on the glycogen index of turkeys at hatch and 7 d posthatch[1]

IOF treatment[2]	Glycogen index[3] Hatch	Day 7
A (protein)	1.27[b]	7.82[a]
B (protein + HMB)	1.23[b]	9.04[a]
C (control)	1.50[b]	7.46[a]
D (HMB)	1.92[a]	7.62[a]
	P-value	
Source of variation		
Protein	0.005	0.277
HMB	0.216	0.397
Protein × HMB	0.144	0.514
SEM (df)	0.150 (36)	0.810 (36)

[a,b]Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]All data represent the mean value ± SE of 10 sample birds per treatment.

[2]Treatment A IOF solution contained 18% EWP in 0.9% NaCl saline. Treatment B IOF solution contained 18% EWP + 0.1% HMB in 0.9% NaCl saline. Treatment C is the noninjected control. Treatment D IOF solution contained 0.1% HMB in 0.9% NaCl saline.

[3]Glycogen index = [total liver glycogen (mg) + total muscle glycogen (mg)]/body mass (g).

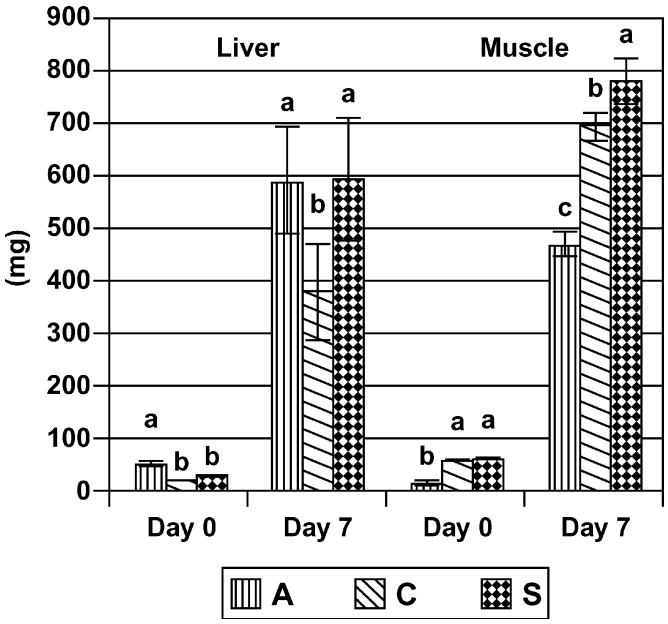

Figure 1. The effects of in ovo feeding (IOF) egg white protein (EWP) and carbohydrates on total liver and pectoralis muscle glycogen of turkeys at hatch and 7 d posthatch. All data represent the mean value ± pooled SE of 10 sample birds per treatment. Treatment A IOF solution contained 18% EWP in 0.9% NaCl saline. Treatment C is the noninjected control. Treatment S contained 20% dextrin + 3% maltose in 0.9% NaCl saline.

[a–c]Means with different superscripts are significantly different ($P < 0.05$).

PC, whereas HMB inclusion in the IO solution enhanced total pectoralis muscle glycogen content relative to the controls. Conversely, IO-fed poults of treatment EWP alone and EWP + HMB had significantly greater pectoral muscle glycogen concentrations in comparison with the control and HMB treatment groups at 7 d posthatch.

Poults IO fed either EWP alone or HMB alone had enhanced LG reserves compared with the controls at hatch (Table 3). Nevertheless, a significant EWP × HMB interaction revealed that the effects of EWP and HMB were not additive as shown by the depressed LG status when these 2 components were combined. By 7 d posthatch, EWP had the main effect on LG reserves. At 1 wk posthatch, there was no EWP × HMB interaction effect on LG status.

Glycogen index, calculated as the sum of the total liver and muscle glycogen divided by body mass (mg/g), is a relative indicator of energy status to support metabolism and growth. Poults IO fed HMB had a significantly higher glycogen index than the control, EWP, or EWP + HMB IO treatments (Table 5). This effect was diminished by 7 d posthatch.

IOF EWP Vs. Carbohydrates

Poults IO fed either the EWP or S treatment had significantly greater BW than the controls at hatch (Table 6). By 3 and 7 d posthatch, there were no significant differences in BW or relative pectoralis mass (data not shown) among the treatments. At hatch, poults IO fed the EWP had significantly greater total LG reserves than the control or S-treatment poults (Figure 1). At 7 d posthatch, poults of EWP and S treatment groups had similar yet significantly greater LG reserves than the controls (Figure 1). Hatchlings IO fed EWP had significantly less total muscle glycogen reserves than the control and S-treated poults. At 7 d posthatch, the S IO-fed poults had significantly greater total muscle glycogen than the control and EWP-treated poults, whereas the EWP-treated poults had significantly less total muscle glycogen than the controls (Figure 1). Poults IO fed S had a significantly higher glycogen index (mg/g) than the noninjected controls and EWP IO fed poults only at 7 d posthatch (Table 7).

DISCUSSION

Main Effects of EWP and HMB Levels

The IOF feeding of EWP had a direct, substrate-mediated effect on LG at hatch and 7 d posthatch. Digested proteins provide free amino acids, the possible substrates for hepatic gluconeogenesis, whereas dietary HMB did not have a substrate-mediated effect of enhancing LG. The primary substrates needed for gluconeogenesis are lactate (Kobayashi et al., 1989) and glucogenic amino acids (Edwards et al., 1997; Watford, 1985) in aves. IOF feeding of HMB enhanced total muscle glycogen reserves at hatch and 7 d posthatch.

The uptake of glucose and amino acids by skeletal muscle is mediated by the action of insulin. 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). Particularly, the amino acid leucine has been shown to be an insulin secretagogue and to potentiate

Table 6. The effects of in ovo feeding (IOF) egg white protein (EWP) and carbohydrates on the BW of turkeys at hatch, 3 and 7 d posthatch (g)[1]

	BW		
IOF treatment[2]	Hatch	Day 3	Day 7
A (protein)	56.02[a]	73.23[a]	131.91[a]
C (control)	52.64[b]	73.64[a]	135.47[a]
S (sugar)	56.65[a]	74.98[a]	139.27[a]
P-value	<0.001	0.634	0.293
SEM (df)	0.265 (176)	0.709 (146)	1.74 (146)

[a,b]Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]All data represent the mean value ± SE of 10 sample birds per treatment.

[2]Treatment A IOF solution contained 18% EWP in 0.9% NaCl saline. Treatment C is the noninjected control. Treatment S contained 20% dextrin + 3% maltose in 0.9% NaCl saline.

glucose-stimulated insulin secretion in pancreatic β-cells (Tsuruzoe et al., 1998). Xu et al. (1998) also demonstrated that the leucine metabolite and precursor to HMB, α-ketoisocaproic acid, is an important signaling mediator in pancreatic β-cell insulin secretion in vitro. While the mechanism of action of HMB has not been identified, we must only speculate the metabolic and biochemical effects of dietary HMB supplementation based upon the current literature. Thus our results imply that IOF of HMB may have stimulated the release of insulin, which resulted in increased uptake of endogenously produced glucose from the liver and the formation of glycogen within the muscles. Hence, energy (glycogen) may be repartitioned from the liver to muscles stores. Therefore, HMB may have an indirect, hormone-mediated effect on total muscle glycogen.

The IOF feeding of HMB alone may have stimulated the release of insulin and insulin-like growth factors. Insulin, an inhibitor of hepatic gluconeogenesis (Pocai et al., 2005) may have metabolically shifted the use of dietary protein (EWP) away from gluconeogenesis. Conversely, these dietary proteins (EWP) 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. Future studies must be conducted to determine the effects of IOF amino acids and their metabolites on carbohydrate metabolism during early avian development.

Table 7. The effects of in ovo feeding (IOF) egg white protein (EWP) and carbohydrates on the glycogen index of turkeys at hatch and 7 d posthatch (g)[1]

	Glycogen index[3]	
IOF treatment[2]	Hatch	Day 7
A (protein)	1.27[a]	7.46[ab]
C (control)	1.50[a]	7.82[b]
S (sugar)	1.59[a]	9.80[a]
P value	0.292	0.081
SEM (df)	0.084 (27)	0.439 (27)

[a,b]Means within a column with different superscripts are significantly different ($P < 0.05$).

[1]All data represent the mean value ± SE of 10 sample birds per treatment.

[2]Treatment A IOF solution contained 18% EWP in 0.9% NaCl saline. Treatment C is the noninjected control. Treatment S contained 20% dextrin + 3% maltose in 0.9% NaCl saline.

[3]Gycogen index = [total liver glycogen (mg) + total muscle glycogen (mg)]/body mass (g).

When EWP and HMB were fed IO together, there were no additive improvements in total liver or muscle glycogen. Several experiments have demonstrated that HMB supplementation increased muscle deposition (Fuller and Nissen, 1994; Flakoll et al., 2004). Thus HMB, when fed in combination with the EWP, may have enhanced the usage of absorbed amino acids for improved muscle deposition and not for use as gluconeogenic precursors for glucose and, ultimately, glycogen formation.

In summary, IOF of EWP + HMB or EWP alone enhanced LG reserves at 7 d posthatch, which may have fueled more rapid growth and development during the early posthatch period. Thus, IOF may serve as an effective method to improve early posthatch growth and muscle deposition, and it may prevent posthatch mortality by enhancing liver and muscle glycogen reserves that can be used to endure energy deficits until sufficient energy is consumed upon the initiation of feed intake.

IOF EWP Vs. Carbohydrates

The IOF feeding of dietary protein enhanced total LG reserves at the day of hatch, whereas dietary carbohydrates fed IO had no effect. Hepatic gluconeogenesis is of primary importance in the carbohydrate metabolism of the avian embryo and neonate (Romanoff, 1967; Donaldson and Christensen, 1992; Christensen et al., 2000). The research reported herein demonstrates the importance of protein substrates for hepatic glucose production. At 7 d posthatch, the glycogen status of poults IO fed proteins and carbohydrates were similar. These results agree with earlier experiments (Romanoff, 1967; Rosebrough et al., 1979) demonstrating that poults and chicks undergo a metabolic shift by 7 d when gluconeogenesis is not the primary mechanism of glucose production because they are nearly adapted to consume a carbohydrate-rich diet. The intake of dietary carbohydrates results in insulin surges that inhibit hepatic gluconeogenesis. Thus after 1 wk of eating a carbohydrate-rich corn diet, the effects of IOF of protein on LG were lost.

Unlike the liver, skeletal muscle requires the action of insulin for the uptake of glucose from the blood. Insulin release occurs with the consumption of a carbohydrate-rich meal and the resultant rise in blood sugar. Thus, PC was not enhanced by IOF of protein, but it was enhanced by IOF of carbohydrates. As expected at d 7, IOF of carbohydrates had a significant effect on muscle glycogen. The IOF of carbohydrates could have resulted in the release of insulin and the uptake and storage of glucose in the form of glycogen in the muscles. Unlike the liver, skeletal muscle lacks the gluconeogenic enzymes needed for the conversion of proteins and amino acids into glucose and therefore would have been unable to use protein IO fed

for enhancement of muscle glycogen stores. The IO feeding of dietary protein targets LG enhancement by taking advantage of high hepatic gluconeogenic rates in the avian neonate; whereas IOF of dietary sugars enhanced muscle glycogen, which ultimately responds as improved BW.

The avian embryo and neonate is similar to an endurance athlete. The endurance athlete maximizes muscle glycogen levels by glycogen loading before an exercise event to help prevent fatigue. Exercise of high intensity and long duration causes depletion of glycogen reserves, which results in fatigue and exhaustion. Additionally, HMB supplementation in trained athletes has been shown to increase muscle mass and strength (Kreider et al., 1999; Panton et al., 2000) and endurance (Vukovich and Dreifort, 2001). Among birds, the hatching process is similar to an exercise event of high intensity and long duration for the endurance athlete. Glycogen stores become depleted during the hatching process due to the high amount of energy expenditure needed. IO feeding of proteins and carbohydrates may help alleviate glucose depletion by glycogen loading before hatch. Thus, the injected nutrients are available for use and storage, which may provide the fuel for hatching and subsequent growth and development.

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REFERENCES

Bakhuis, W. L. 1974. Observations on hatching movements in chick (*Gallus domesticus*). J. Comp. Physiol. Psychol. 87:997–1003.

Christensen, V. L., and W. E. Donaldson. 1992. Effect of oxygen and maternal dietary iodine on embryonic carbohydrate metabolism and hatchability of turkey eggs. Poult. Sci. 71:747–753.

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

Christensen, V. L., M. J. Wineland, G. M. Fasenko, and W. E. Donaldson. 2001. Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. Poult. Sci. 80:1729–1735.

Donaldson, W. E., and V. L. Christensen. 1992. Effects of injections on L-alanine, L-glucose and L-ascorbic acid in newly hatched turkey poults on glucose metabolism. Comp. Biochem. Physiol. 101:849–852.

Dreiling, C. E., D. E. Brown, L. Casale, and L. Kelly. 1987. Muscle glycogen: Composition of iodine binding and enzyme digestion assays and application to meat samples. Meat Sci. 20:167–177.

Edwards, H. M., III, D. H. Baker, S. R. Fernandez, and C. M. Parsons. 1997. Maintenance threonine requirement and efficiency of its use for accretion of whole-body threonine and protein in young chicks. Br. J. Nutr. 78:111–119.

Flakoll, P., R. Sharp, S. Baier, D. Levenhagen, C. Carr, and S. Nissen. 2004. Effect of beta-hydroxy-beta-methylbutyrate, arginine, and lysine supplementation on strength, functionality, body composition, and protein metabolism in elderly women. Nutrition 20:445–451.

Freeman, B. M. 1965. The importance of glycogen at the termination of the embryonic existence of *Gallus domesticus*. Comp. Biochem. Physiol. 14:217–222.

Freeman, B. M. 1969. The mobilization of hepatic glycogen in the *Gallus domesticus* at the end of incubation. Comp. Biochem. Physiol. 28:1169–1176.

Freeman, B. M., and A. C. Manning. 1971. Glycogenolysis and lipolysis in *Gallus domesticus* during the perinatal period. Comp. Gen. Pharmacol. 2:198–204.

Fuller, J. C., and S. Nissen. 1994. Decreasing male broiler mortality by feeding the leucine catabolite β-methyl-β- hydroxybutyrate. Poult. Sci. 73(Suppl. 1):93. (Abstr.)

George, J. C., and P. T. Iype. 1963. The mechanism of hatching in the chick. Pavo 1:52–56.

John, T. M., J. C. George, and E. T. Moran, Jr. 1987. Pre- and post-hatch ultra-structural and metabolic changes in the hatching muscle of turkey embryos from antibiotic and glucose-treated eggs. Cytobios 49:197–210.

John, T. M., J. C. George, and E. T. Moran, Jr. 1988. Metabolic changes in pectoral muscle and liver of turkey embryos in relation to hatching: Influence of glucose and antibiotic treatment of eggs. Poult. Sci. 67:463–469.

Kobayashi, T., H. Iwai, R. Uchimoto, M. Ohta, M. Shiota, and T. Sugano. 1989. Gluconeogenesis in perfused livers from dexamethasone-treated chickens. Am. J. Physiol. 256:R907–R914.

Kreider, R. B., M. Ferreira, M. Wilson, and A. L. Almada. 1999. Effects of calcium beta-hydroxy-beta-methylbutyrate (HMB) supplementation during resistance-training on markers of catabolism, body composition and strength. Int. J. Sports Med. 20:503–509.

Moran, E. T., Jr. 1988. Subcutaneous glucose is more advantageous in establishing the post-hatch poult than oral administration. Poult. Sci. 67:493–501.

Moran, E. T., Jr. 1989. Effects of post-hatch glucose on poults fed and fasted during yolk sac depletion. Poult. Sci. 68:1141–1147.

Moran, E. T., Jr. 1990. Effects of egg weight, glucose administration at hatch, and delayed access to feed and water on the poult at 2 weeks of age. Poult. Sci. 69:1718–1723.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Sci. Washington, DC.

Panton, L. B., J. A. Rathmacher, S. Baier, and S. Nissen. 2000. Nutritional supplementation of the leucine metabolite beta-hydroxy-beta-methylbutyrate (HMB) during resistance training. Nutrition 16:734–739.

Pocai, A., T. K. Lam, R. Gutierrez-Juarez, S. Obici, G. J. Schwartz, J. Bryan, L. Aguilar-Bryan, and L. Rossetti. 2005. Hypothalamic K (ATP) channels control hepatic glucose production. Nature 434:1026–1031.

Romanoff, A. L. 1967. Chemistry of the non-embryonic portions of eggs. Pages 177–232 in Biochemistry of the Avian Embryo: A Qualitative Analysis of Prenantal Development. Interscience Publ., New York, NY.

Rosebrough, R. W., E. Geis, K. Henderson, and L. T. Frobish. 1979. Control of glycogen metabolism in the developing turkey poult. Growth 43:188–201.

SAS Institute. 1996. SAS User's Guide. Version 6. SAS Inst. Inc., Cary, NC.

Starck, J. M., and R. E. Rickelefs. 1998. Energy metabolism, gas exchange, and ventiliation. Pages 89–111 in Avian Growth and Development: Evolution Within the Altricial-Precocial Spectrum. Oxford Univ. Press, Oxford, UK.

Tsuruzoe, K., E. Araki, N. Furukawa, T. Shirotani, K. Matsumoto, K. Kaneko, H. Motoshima, K. Yoshizato, A. Shirakami, H. Kishikawa, J. I. Miyazaki, and M. Shichiri. 1998. Creation and characterization of a mitochondrial DNA-depleted pancreatic beta-cell line: Impaired insulin secretion induced by glucose, leucine, and sulfonylureas. Diabetes 47:621–631.

Uni, Z., and P. Ferket. 2003. Enhancement of development of oviparous species by in ovo feeding. US Regular Patent US 6,592,878 B2. North Carolina State Univ., Raleigh and Yissum Res. Dev. Co., Hebrew Univ. Jerusalem, Israel.

Uni, Z., and P. R. Ferket. 2004. Methods for early nutrition and their potential. World's Poult. Sci. J. 60:101–111.

Uni, Z., P. R. Ferket, E. Tako, and O. Kedar. 2005. In ovo feeding improves energy status of late-term chicken embryos. Poult. Sci. 84:764–770.

Vukovich, M. D., and G. D. Dreifort. 2001. Effect of beta-hydroxy beta-methylbutyrate on the onset of blood lactate accumulation and V(O)(2) peak in endurance-trained cyclists. J. Strength Cond. Res. 15:491–497.

Warriss, P. D., S. C. Kestin, S. N. Brown, and E. A. Bevis. 1988. Depletion of glycogen reserves in fasting broiler chickens. Br. Poult. Sci. 29:149–154.

Watford, M. 1985. Gluconeogenesis in the chicken: Regulation of phosphoenolpyruvate carboxykinase gene expression. Fed. Proc. 44:2469–2474.

Xu, G., G. Kwon, C. A. Marshall, T. A. Lin, J. C. Lawrence, and M. L. McDaniel. 1998. Branched-chain amino acids are essential in the regulation of PHAS-I and p70 S6 kinase by pancreatic β-cells. J. Biol. Chem. 273:28178–28184.