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# The Effects of Amniotic Nutrient Administration, "*In ovo* Feeding" of Arginine And/or ß-Hydroxy-ß-Methyl Butyrate (HMB) on Insulin-like Growth Factors, Energy Metabolism and Growth in Turkey Poults

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**Abstract:** *In ovo* feeding (IOF), injecting nutrients into the amnion, may improve growth performance by enhancing circulating IGF's and glycogen reserves. To test this hypothesis 400 Hybrid® turkey eggs were injected into the amnion with 1.5 ml saline solutions consisting of 4 IOF formulation treatments consisting of a factorial arrangement of 2 levels of arginine (ARG 0 or 0.7%) and 2 levels of HMB (0 or 0.1%) at 23 days (d) of incubation. At hatch, poults were fed *ad libitum* and bodyweights (BW), organ weights, total liver and pectoralis muscle (PM) glycogen were taken at hatch 3, 7, 10, and 14 d. Additionally, hepatic glucose-6-phosphatase (G6P) activity was determined at hatch and 7 d. Heparinized blood samples were taken at hatch, 3 d, 7 d and 14 d and plasma was analyzed for IGF-I and IGF-II levels using an acid-ethanol extraction method. Although arginine had no affect on BW, there were significant main effects of HMB on increased BW from hatch through 14 d. Plasma IGF-I levels were significantly enhanced at hatch, 3 and 7 d in poults *in ovo* fed both ARG and HMB, but not when either factor was independent. Significant ARG X HMB effects were observed on IGF-II at hatch, 3d and 7d: without ARG, IGF-II was decreased by HMB, but it increased when added with ARG. All *in ovo* treatments increased G6P at hatch, while, G6P was depressed by HMB or ARG alone at 7 d. Total hepatic glycogen was increased only at hatch by HMB or ARG, but their effects were not additive as indicated by a significant ARG X HMB effect. *In ovo* administration of HMB and arginine enhances hepatic liver reserves, which may provide the fuel needed for rapid subsequent growth during the critical post-hatch period.

**Key words:** *in ovo* feeding, arginine, ß-hydroxy-ß-methyl butyrate, glycogen, IGF's, turkey

## Introduction

The avian embryo develops in a carbohydrate-free environment with a finite amount of *in ovo* (IO) energy and nutrients to support embryonic growth and hatching. As the embryo grows and develops IO, the yolk reserves and albumen decline transiently and are nearly depleted prior to hatching. The yolk lipids and albumen protein provide the gluconeogenic substrates for endogenous production of glucose, the primary fuel, stored as glycogen in the liver and muscles. Therefore, glycogen reserves provide the fuel needed for growth, development and hatching and are critical for the survival of the avian embryo and neonate. At hatch, glycogen reserves can be replenished with the immediate access and consumption of external feed. In commercial practice however, immediate access to feed is often delayed for 24-48 hours after hatch (Moran and Reinhart, 1980) and hatchlings must mobilize critical body resources (primarily muscle) to provide the energy needed for maintenance, thermoregulation and activity. Poor nutritional status of the avian neonate during this critical post-hatch period has been shown to increase mortality rates (Noble *et al*., 1986), permanently stunt growth (Vieira and Moran, 1999) and decrease muscle deposition (Modziak *et al*., 1997; Halevy *et al*., 2000). Additionally, Kita *et al*. (2002) demonstrated that food deprivation reduces plasma IGF-I levels by 50% in comparison to conventional chicks.

*In ovo* feeding (Uni and Ferket, 2003) is the administration of exogenous nutrients into the amnion of the late-term avian embryo. Because the embryo orally consumes the amniotic fluid (primarily water and albumen protein) prior to pipping of the air cell, supplementing the amnion with nutrients is fundamentally feeding the embryo an external diet prior to hatch. Hence *in ovo* feeding (IOF) may serve as a tool to overcome early growth constraints during embryonic and post-hatch development in domestic poultry.

Uni and Ferket (2003) demonstrated that turkey poults *in ovo* fed about 1 mg HMB, a leucine metabolite, had increased in hepatic glycogen by about 40% over saline-injected and non-injected controls. Moreover, hatchability rates were positively correlated with liver glycogen content of turkey and chick embryos before hatch (Uni and Ferket, 2003). *In ovo* feeding of carbohydrates and protein (Uni and Ferket, 2004) or carbohydrates and/or

HMB (Uni *et al.*, 2005) increased broiler hatching bodyweights, relative pectoralis breast muscle and improved hepatic glycogen reserves over the controls.

Arginine (ARG) may also be a candidate nutrient for *in ovo* feeding based on research results reported by other researchers. Chevalley *et al.* (1998) demonstrated that pharmacological doses of ARG *in vitro* enhanced collagen and bone formation by stimulating IGF-I production in osteoblasts. Kita *et al.* (2002) reported that dietary supplementation of a high protein diet with ARG, methionine and cysteine enhanced plasma IGF-I levels and bodyweight gain in young chicks. Kim *et al.* (2004) reported that dietary ARG supplementation significantly increased plasma concentrations of insulin and growth hormone by 24-27% and enhanced the average daily weight gain by 28-66% over the controls in artificially reared piglets. Flakoll *et al.* (2004) observed that dietary supplementation of HMB, ARG and lysine significantly improved limb circumference, marked by a 20% increase in protein synthesis and strength in 23 elderly women in comparison to the placebo group.

Based on their metabolic effects on growth and development, HMB and ARG are possible candidate nutrients for *in ovo* feeding technology because they may work independently or in combination to enhance glycogen accretion during late-term embryonic development, and muscle deposition and early growth of turkey poults during the critical post-hatch period. We hypothesize that *in ovo* feeding ARG and/or HMB may increase the hepatic gluconeogenic enzymes to promote hepatic glycogen accretion, which can support the rapid post-hatch growth. *In ovo* feeding of ARG and/or HMB may also enhance plasma IGF's and subsequently enhance somatic growth and development. Similar to other species, IGF-I plays a very important role in growth, metabolism and development in birds (King and Scanes, 1986) and enhanced levels may improve early growth and muscle deposition.

## Materials and Methods

**ARG and HMB IOF dose response experiment:** A dose response ARG and HMB IOF trial was conducted to determine the optimal levels of ARG and HMB conducive to increased growth in turkeys. The National Research Council (NRC, 1994) recommends that at hatch young poults should be fed a diet containing 1.6% ARG. Based on this dietary ARG requirement, the ARG content levels of the *in ovo* feeding solutions were selected for testing, by either increasing or decreasing in dosage by a factor of two. Twenty eggs per treatment were injected with 1.5mL of 0.9% saline of the following ARG levels: 0%, 0.2%, 0.7%, 7.7%, 15.4%, 23.0%, and 30.7% at 23 days of embryonic development. Additionally, 20 eggs per treatment were injected with 1.5mL of 0.9% saline with graded levels of HMB based upon previous feeding trials conducted by Nissen et al (1994) and Uni and Ferket (2004) (0%, 0.1%, 0.3%, 1.0%, 3.0%). The bodyweights (BW) were recorded at hatch, 3, 5, 8, 10, 14, 21, and 28 days post-hatch.

**Incubation and *in ovo* feeding (IOF):** Hybrid® turkey embryos were obtained at 19 days of incubation from a commercial hatchery (Prestage Farms, Clinton, NC) and their incubation was continued according to standard hatchery practices (99.9-100.0°F). Twenty eggs per treatment were placed within one of four trays, such that each treatment was equally distributed within the incubator. At 21 days of incubation, 400 eggs were individually weighed and distributed among four 5-gram weight categories ranging from 65-70g, 71-75g, 76-80g, 81-85g. These eggs were evenly distributed among four treatment groups of 100 eggs each, such that the weight distribution profile among all 4 treatment groups was identical. At 23 days of incubation, each egg was candled to identify the location of the amnion. A targeted hole was punched using a 23 gauge needle and 1.5 ml of IOF solution was injected into the amnion to a depth of about 15 mm. The IOF injection site was disinfected with ethyl alcohol, sealed with cellophane tape, and transferred to hatching baskets. The IOF test solutions were aseptically prepared to contain 0.1% HMB[1] in 0.4% saline, 0.7% ARG[2] in 0.4% saline; or 0.1% HMB + 0.7% ARG in 0.4% saline. These treatments were compared to non-injected controls that were subjected to the same handling procedures as the IOF treatment groups. A preliminary experimentation in our laboratory demonstrated that *in ovo* injection of 2.0 mL of 0.9% saline did not affect embryo and poult bodyweights, breast yield or glycogen status. Therefore our experimental design allowed us to statistically evaluate IOF solutions containing a factorial arrangement of two levels of HMB (0 and .1%) and two levels or ARG (0 and 0.7%).

**Animal husbandry and tissue sampling:** Upon hatching, each poult was marked for identification and the BW was recorded at hatch, 3, 7, 10 and 14 days post-hatch. Hatchability for all treatments was equal to or greater than 96%. Twenty-five poults from each IOF treatment were assigned randomly to each of four rooms of approximately two hundred square feet each with approximately two square feet per bird. Heat was provided by propane heaters to maintain room temperatures of about 27°C, and supplemental incandescent heat lamps were used to maintain a brooding temperature of approximately 40°C. The concrete floor of each room was covered with a 10 cm layer of soft pine shavings as litter. Each room was also equipped with automatic plasmon drinkers and a typical starter feed (2935 kcal/kg, 27.5% protein, and 5.6% fat) was provided *ad libitum* in small tube feeders. Poults were maintained on 12 h light (10 lux) and 12 hr dark. At

hatch, 3 d, 7 d and 10 d post-hatch, 10 poults were randomly selected from each treatment (i.e. ~2 poults/room/treatment) and sampled for plasma IGF-I and IGF-II determination, liver glycogen content and glucose-6-phosphate activity, and breast muscle glycogen. All experimental protocols were approved by the Institutional Animal Care and Use Committee at North Carolina State University.

**Tissue sampling and glycogen analysis:** At 25 days of incubation, (48 hours post-IOF), the pectoralis muscle and liver from turkey embryos were weighed and stored at -20°C for glycogen analysis. Additionally, at hatch, 3, 7 and 14 days post-hatch, 10 birds per treatment were euthanized by cervical dislocation and within 2 minutes the whole liver and pectoralis muscle was dissected and placed in an ice bath 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 to account for errors associated with glycogen analysis. The pectoralis muscle and liver samples were homogenized in 8% perchloric acid (1g/4mL), 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 minutes. 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 minutes. Subsequently, a 10uL aliquot of sample (from the bottom layer) was added to a disposable cuvette, along with 0.4mL of 8% perchloric acid and 2.6mL of iodine color reagent made of 1.3 mL of solution A (0.26g iodine + 2.6g 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 460nm. The amount of glycogen present in a 10uL sample is determined by preparation of a known glycogen standard curve.

**Hepatic glucose-6-phosphatase analysis:** Hepatic glucose-6-phosphatase activity was determined using modified procedures by Donaldson and Christensen (1991). Due to a limited availability of tissues, only liver samples taken at hatch and 7 days post-hatch were homogenized in a 0.25M sucrose solution (1g liver/10 mL) and centrifuged at 14,000 rpm at 4°C for 10 minutes. The supernatant was diluted 1:4 with 0.25M sucrose solution. Three tubes were prepared for each experimental sample containing the following: 0.3mL of 0.1% histidine solution, 0.1 mL 0.25M sucrose solution, and 0.1mL of diluted sample. At 15 second intervals each tube was placed into a 37°C water bath for a total incubation time of 10 minutes. A volume of 0.1mL of 0.4M glucose-6-phosphate solution was added to two tubes of the triplicate set prior to incubation, and the remaining tube of the triplicate served as a sample blank. After 10 minutes of incubation, 1mL of 10% trichloroacetic acid (TCA) was added to each tube at 15 second intervals. For the sample blanks, 0.1 mL of 0.4M glucose-6-phosphate solution was added to each tube. Subsequently, the inorganic phosphate levels were determined for each sample. A separate set of tubes were prepared for each sample in triplicate, plus an additional tube for a standard. Two milliliters of a 40% ferrous sulfate solution was added to each tube. In each corresponding tube, 1mL of sample was added, with each sample blank receiving 1mL of 5% TCA. In the standard tube, 0.6mL of 5% TCA and 0.4mL of 1.25mM phosphate standard was added in 5% TCA. All the tubes were mixed and allowed to stand for at least 10 minutes (but less than 60 minutes) before reading optical density (OD) at 700nm.

**Procedure for protein determination:** Protein was determined using the Lowry method described by the BioRad ®Protein Assay kit with Bovine Serum Albumin Assay Standard II using reagents #500-0007, #500-0115, #500-0114 and #500-0113 (BioRad®, Hercules, CA; www.bio-rad.com).

Plasma IGF-I and IGF-II Analysis: Blood samples were taken from the jugular vein with heparinized syringes from 10 birds per treatment at hatch, 3, 7 and 14 days post-hatch prior to cervical dislocation. Blood samples were spun by centrifugation to collect the plasma. Plasma IGF-I and IGF-II measurements were performed using acid-ethanol extraction as described by McMurtry *et al.* (1994, 1998). The intra-assay coefficient of variation was 3.1% and 4.2% for IGF-I and IGF-II assays, respectively

**Statistical analysis:** All data were statistically analyzed using general linear models procedures for ANOVA (SAS, 1996) with a completely randomized design. Each bird served was an experimental unit for statistical analysis. Data were analyzed as a 2 X 2 factorial arrangement of two levels of ARG (0% and 0.7%) and two levels of HMB (0% and 0.1%). All data were sorted by age and treatment within the model. Means were separated by least-squares-means in SAS (1996) and the treatment effects were considered significant at $P<0.05$. All experiments were conducted with an equal frequency of variables within each treatment.

## Results

**HMB and ARG IOF dose response:** Bodyweights for the non-injected and the saline-injected controls were similar at the time points measured (data not shown). Hence, bodyweights for the non-injected controls were used for comparisons, as a normal practice. The IOF ARG levels of 7.7% or greater were lethal to the embryo

Table 1: The effects of IOF of arginine (ARG) and Beta-hydroxy- Beta-methylbutyrate (HMB) on the body weights of turkeys at hatch, 3, 7, 10 and 14 days post-hatch[1]

| IOF Treatment | | Days of Age | | | | |
|---|---|---|---|---|---|---|
| ARG | HMB | Hatch | Day 3 | Day 7 | Day 10 | Day 14 |
| | | (g) | | | | |
| 0 | 0 | 64.5[b] | 82.0[b] | 136.2 | 164.4 | 230.2[b] |
| 0 | 0.1% | 67.2[a] | 85.9[a] | 139.7 | 179.6 | 253.0[ab] |
| 0.7% | 0 | 66.7[a] | 84.3[ab] | 134.1 | 172.6 | 236.6[b] |
| 0.7% | 0.1% | 67.5[a] | 86.4[a] | 140.3 | 182.6 | 257.7[a] |
| Source of Variation | | P-value | | | | |
| ARG | | 0.061 | 0.227 | 0.950 | 0.273 | 0.471 |
| HMB | | 0.008 | 0.011 | 0.020 | 0.014 | 0.005 |
| ARG X HMB | | 0.173 | 0.436 | 0.840 | 0.607 | 0.912 |
| SEM (DF)[2] | | 0.63 (267) | 1.34 (233) | 2.64 (170) | 4.68 (138) | 6.21 (141) |

[1]All data represents the mean of 25 replicate birds per treatment. [2]SEM (DF) = pooled standard error of the mean and (degrees of freedom). [a,b]Means within a column with different superscripts are significantly different (P<0.05).

upon IO administration. Poults IO fed 0.2% ARG had bodyweights that were 3%, 18%, 24% and 15% greater than the controls at days 10, 14, 21 and 28 days post-hatch, while poults IO fed 0.7% ARG had bodyweights that were 1%, 2%, 5%, 7%, 17%, 9% and 3% greater than the controls at days 3, 5, 8, 10, 14, 21, and 28 days post-hatch, respectively (Fig. 1). Hatchability of 0.2% and 0.7% ARG IO fed poults was 73% and 91%, respectively (data not shown). Therefore 0.7% ARG was identified as an optimal level for IOF due to improved hatchability and bodyweights in comparison to the 0.2% ARG IOF group. Hatchability was similar (greater than 90%) for all poults IO fed graded levels of HMB (data not shown). Poults IO fed 0.1% HMB had the greatest improvements in bodyweight gains of 7.0% 27% between 8- 14 days post-hatch relative to the non-injected controls (Fig. 1). Therefore, due to a high hatchability rate and improvements in bodyweight gains 0.1% HMB was identified as an optimal level for IOF. Hatchability was 82% for the non-injected controls and 61% for the 0.9% saline injected controls (data not shown), while bodyweights were similar at all time points measured. Therefore, 0.4% saline was utilized instead of 0.9% saline as the IOF carrier for all subsequent IOF experiments.

**The effects of IOF of two levels of ARG (0% and 0.7%) and two levels of HMB (0% and 0.1%):** Hatchability for all treatments was equal to or greater than 96%, while mortality rates were less than 1% through the 14 days post-hatch. There was no significant ARG X HMB interaction on BW at hatch, 3, 7, 10 or 14 d post-hatch. Although there was a marginal enhancement of BW by IOF ARG at hatch, there was a significant main effect of HMB on BW from hatch to 14 d of age, and this effect increased with age as indicated by a significant age X HMB effect on BW (Table 1: F=2.57, P=0.0397). Therefore, the inclusion of HMB in the IOF solutions significantly enhanced bodyweights at hatch, 3 and 14 d post-hatch. All IOF poults were 3-4% heavier (P=0.0161) at hatch than controls (Table 1). At 3 d post-hatch, poults IOF HMB (HMB alone or in combination with ARG) had BWs that were 4.2%-5.1% greater than the controls (P=0.0574). Additionally, the poults *in ovo* fed HMB and ARG were 10.2% (P=0.0786) and 10.7% (P=0.0287) heavier than the controls at day 10 and 14 post-hatch, respectively (Table 1). There were no significant effects on relative pectoralis or liver mass at any of the time points measured (data not shown).

There were significant ARG X HMB interaction effects on plasma IGF-I levels at hatch, 3 and 7 post-hatch (Table 2), while there was a highly significant ARG X HMB interaction effect (P<0.01) on plasma IGF-II levels at hatch and 7 d post-hatch and a marginal effect at 3 d (P<0.10) (Table 3). It is noteworthy that there were no main effects of HMB and ARG on plasma IGF-I and IGF-II, with the exception of a HMB main effect on plasma IGF-I at 7d post-hatch. At hatch, plasma IGF-I (P=0.0344) and IGF-II (P=0.0072) levels were significantly enhanced by IOF .7% ARG with .1% HMB in comparison to all other treatments. This interaction effect was lost by 3 d post-hatch with only marginal enhancements of plasma IGF-I levels in poults IOF a combination of ARG and HMB. At 7 d post-hatch, *in ovo* feeding either HMB or ARG alone significantly depressed plasma IGF-II levels (P=0.0009); while this effect was lost by 14 d post-hatch (Table 3).

As expected, there were highly significant age effects on total hepatic glycogen (Table 4) and total pectoralis muscle glycogen (Table 5), glycogen status index ((mg total liver glycogen + mg muscle glycogen)/ g BW) (Table 6), and hepatic G6P activity (Table 7) (P<0.001). There were significant ARG and a HMB X ARG interaction effects on total hepatic glycogen at hatch, while these effects were absent at all other time points. Poults of the IOF treatments containing ARG, HMB and HMB + ARG had, a 84%, 78.7%, and 75% increase in total hepatic glycogen content at the day of hatch in comparison to the controls, respectively, (P=0.0071, Table 4). While the hepatic glycogen status index of poults *in ovo* fed

Table 2: The effects of *in ovo* feeding (IOF) of arginine (ARG) and Beta-hydroxy- Beta-methylbutyrate (HMB) on the plasma IGF-I of turkeys at hatch, and 3, 7 and 14 days post-hatch[1]

| IOF Treatment | | Days of Age | | | |
|---|---|---|---|---|---|
| ARG | HMB | Hatch | Day 3 | Day 7 | Day 14 |
| | | (ng/mL) | | | |
| 0 | 0 | 18.10[b] | 12.89 | 12.57 | 17.30 |
| 0 | 0.1% | 15.57[b] | 11.24 | 11.76 | 18.23 |
| 0.7% | 0 | 17.41[b] | 11.27 | 10.50 | 16.75 |
| 0.7% | 0.1% | 26.56[a] | 14.52 | 12.54 | 18.00 |
| Source of Variation | | P-value | | | |
| ARG | | 0.071 | 0.313 | 0.807 | 0.879 |
| HMB | | 0.239 | 0.588 | 0.035 | 0.648 |
| ARG X HMB | | 0.042 | 0.041 | 0.014 | 0.941 |
| SEM (36)[2] | | 2.79 | 1.00 | 0.600 | 2.40 |

[1]All data represents the mean of 10 replicate birds per treatment. [2] SEM (36) = pooled standard error of the mean and 36 degrees of freedom. [a,b]Means within a column with different superscripts are significantly different (P<0.05).

Table 3: The effects of *in ovo* feeding (IOF) of arginine (ARG) and Beta-hydroxy- Beta-methylbutyrate (HMB) on the plasma IGF-II of turkeys at hatch, and 3, 7 and 14 days post-hatch[1]

| IOF Treatment | | Days of Age | | | |
|---|---|---|---|---|---|
| ARG | HMB | Hatch | Day 3 | Day 7 | Day 14 |
| | | (ng/mL) | | | |
| 0 | 0 | 64.10[ab] | 31.89 | 40.31[a] | 56.38 |
| 0 | 0.1% | 47.47[b] | 23.65 | 24.98[b] | 56.93 |
| 0.7% | 0 | 55.34[b] | 21.85 | 23.54[b] | 53.10 |
| 0.7% | 0.1% | 80.75[a] | 32.89 | 37.45[a] | 61.41 |
| Source of Variation | | P-value | | | |
| ARG | | 0.104 | 0.648 | 0.512 | 0.946 |
| HMB | | 0.554 | 0.823 | 0.828 | 0.581 |
| ARG X HMB | | 0.007 | 0.097 | <0.001 | 0.430 |
| SEM (36)[2] | | 7.49 | 4.20 | 3.20 | 6.10 |

[1]All data represents the mean of 10 replicate birds per treatment. [2]SEM (36) = pooled standard error of the mean and 36 degrees of freedom. [a,b]Means within a column with different superscripts are significantly different (P<0.05).

solutions containing HMB were similar, both were significantly greater than the controls at hatch, and significantly less than the hepatic glycogen status of IOF poults provided ARG (Table 6). Conversely, there were no IOF treatment effects on total muscle glycogen at any of the time points measured (Table 5).

Glycogen status index is a relative indicator of energy status to support metabolism and growth. All *in ovo* fed poults had significantly greater glycogen index at hatch in comparison to the controls (Table 6), with main effects of ARG (P = 0.009). By 3 d post-hatch, there were no significant differences in the glycogen index between the IOF treatments. Additionally, poults IO fed HMB, ARG, and HMB + ARG had significantly greater hepatic G6P activity at hatch over the controls (P=0.0001, Table 7), with a HMB X ARG interaction (P<0.05). At 7 days post-hatch, hepatic G6P activity of poults IOF ARG with or without HMB were similar to the controls, while G6P activity was significantly lower in poults IOF HMB alone (P=0.0143).

## Discussion

Our results demonstrate that embryonic administration (*in ovo* feeding) of HMB and/or ARG alone or in combination enhances plasma IGF-I, IGF-II levels, hepatic glucose-6-phosphatase activity and total hepatic glycogen at hatch in comparison to conventional hatchlings, which fueled more rapid subsequent growth during the early post-hatch period. Nutritional status influences overall body and muscle growth and may do so directly or indirectly through its effect on regulatory factors, such as circulating IGF-I (Brameld, 1997; Beccavin *et al.*, 2001; Roberson *et al.*, 2002). During embryonic development, there is a steady rise in IGF-I from day 6 to peak at day 15 of incubation, and then declines to low levels at hatch (Robcis *et al.*, 1991).

The first meal after hatch is critical for growth and development of young hatchlings. In commercial hatcheries hatchlings may be without food and water for 48-72 hours after hatch (Moran and Reinhart, 1980), which may delay enteric development (Yamauchi *et al.*, 1996; Geyra *et al.*, 2001, 2002) muscle deposition (Mozdziak *et al.* 1997; Halevy *et al.*, 2000) and body growth. Thus, in the conventional hatchling (fasting), circulating IGF-I and IGF-II levels are reduced (Beccavin *et al.*, 2001), and may have detrimental effects on the post-hatch growth and development. Conversely, our results demonstrate that poults *in ovo* fed a combination

Table 4: The effects of IOF of arginine (ARG) and Beta-hydroxy- Beta-methylbutyrate (HMB) on total hepatic glycogen of turkeys at 25 days of incubation and at hatch, 3, 7 and 14 days post-hatch[1]

| IOF treatment | | Days of Age | | | |
|---|---|---|---|---|---|
| ARG | HMB | Hatch | Day 3 | Day7 | Day14 |
| | | (mg) | | | |
| 0 | 0 | 12.1[c] | 499.7 | 38.8 | 1262.4 |
| 0 | 0.1% | 54.7[b] | 684.0 | 33.2 | 2229.5 |
| 0.7% | 0 | 77.0[a] | 661.2 | 39.0 | 1769.3 |
| 0.7% | 0.1% | 48.5[b] | 534.3 | 51.6 | 987.4 |
| Source of Variation | | P-Value | | | |
| ARG | | 0.024 | 0.951 | 0.377 | 0.500 |
| HMB | | 0.574 | 0.764 | 0.737 | 0.865 |
| ARG X HMB | | 0.007 | 0.111 | 0.384 | 0.114 |
| SEM (36)[2] | | 6.21 | 47.6 | 5.12 | 266.2 |

[1]All data represents the mean value of 10 sample birds per treatment. [3]SEM (36) = pooled standard error of the mean with 36 degrees of freedom. [a,b]Means within a column with different superscripts are significantly different (P<.0.05)

Table 5: The effects of *in ovo* feeding (IOF) of arginine (ARG) and Beta-hydroxy- Beta-methylbutyrate (HMB) on pectoralis muscle glycogen of turkeys at 25 days of incubation and at hatch, 3, 7 and 14 days post-hatch[1]

| IOF treatment | | Days of Age | | | |
|---|---|---|---|---|---|
| ARG | HMB | Hatch | Day 3 | Day 7 | Day 14 |
| | | (mg) | | | |
| 0 | 0 | 5.88 | 24.5 | 30.8 | 192.8 |
| 0 | 0.1% | 6.94 | 29.2 | 22.7 | 200.2 |
| 0.7% | 0 | 5.19 | 28.1 | 25.7 | 214.1 |
| 0.7% | 0.1% | 6.40 | 25.1 | 33.4 | 213.7 |
| Source of Variation | | P-Value | | | |
| ARG | | 0.47 | 0.94 | 0.54 | 0.66 |
| HMB | | 0.18 | 0.80 | 0.97 | 0.93 |
| ARG X HMB | | 0.93 | 0.24 | 0.89 | 0.92 |
| SEM (36)[2] | | 0.418 | 1.64 | 2.26 | 19.30 |

[1]All data represents the mean of 10 sample birds per treatment. [2]SEM (36) = pooled standard error of the mean with 36 degrees of freedom. [a,b]Means within a column with different superscripts are significantly different (P<.05).

of HMB and ARG had significantly increased circulating IGF-I and IGF-II levels at the day of hatch in comparison to the controls, which parallel the improved bodyweight gains through 14 days post-hatch. Several studies have demonstrated that increased circulating IGF-I levels increase intestinal glucose absorption (Zhang *et al.*, 1995, Alexander and Carey, 1999, Lane *et al.,* 2002) and may subsequently be stored as glycogen in the liver and muscles (by the action of insulin). Thus, IOF of HMB + ARG may modulate carbohydrate metabolism indirectly through the action of insulin and/or IGF-1. Our results support this hypothesis, as poults IOF ARG (ARG alone or in combination with HMB) had enhanced total hepatic glycogen reserves and glycogen index at hatch. Gluconeogenesis is the primary mechanism of glucose production in the avian embryo and neonate (Romanoff, 1967). The avian embryo develops in virtually a carbohydrate-free environment and must rely upon gluconeogenesis for the production of glucose, the primary fuel for growth and development. Developmentally, the gluconeogenic pathways are highly active and decrease after hatching when the chicks are fed a high carbohydrate diet (Nelson *et al.,* 1966, Wallace and Newsholme, 1967). Glucose-6-phosphatase is one of the key gluconeogenic enzymes required for the conversion of proteins and amino acids into glucose. Our experimental results parallel these earlier findings, with a precipitous temporal decline in hepatic glucose-6-phosphatase activity from the day of hatch to 7 d post-hatch in all hatchlings.

We demonstrate that *in ovo* fed poults have a higher gluconeogenic activity at hatch than the controls, as indicated by enhanced hepatic G6P activity. Hence, *in ovo* feeding may increase the late-term embryo's capacity to convert gluconeogenic precursors (yolk lipids, proteins, amino acids) into glucose, for storage in the liver and muscles as glycogen and may directly enhance hepatic glycogen accretion. While there were no statistical differences (P<0.05) in hepatic glycogen status among IOF poults at 3 d post-hatch, poults *in ovo* fed HMB or ARG alone had approximately a 26% greater glycogen reserves than did the controls, while those provided IOF HMB + ARG had 11% greater glycogen reserves than the controls. Therefore, hepatic glycogen reserves may have been enhanced directly by *in ovo* feeding due to increased hepatic gluconeogenic activity (measured as glucose-6-phosphatase activity) and enhanced indirectly due to enhanced circulating IGFs.

Table 6: The effects of *in ovo* feeding (IOF) of arginine (ARG) and Beta-hydroxy- Beta-methylbutyrate (HMB) on the glycogen index of turkeys at 25 days of incubation and at hatch, 3, 7 and 14 days post-hatch[1]

| IOF treatment | | Days of Age | | | |
|---|---|---|---|---|---|
| ARG | HMB | Hatch | Day 3 | Day 7 | Day 14 |
| | | Glycogen Status Index[2] | | | |
| 0 | 0 | 0.29[c] | 6.58 | 0.55 | 6.31 |
| 0 | 0.1% | 0.81[b] | 9.32 | 0.43 | 9.41 |
| 0.7% | 0 | 1.22[a] | 8.91 | 0.49 | 8.20 |
| 0.7% | 0.1% | 0.84[b] | 6.87 | 0.52 | 4.25 |
| Source of Variation | | P-value | | | |
| ARG | | 0.009 | 0.961 | 0.275 | 0.460 |
| HMB | | 0.677 | 0.780 | 0.69 | 0.848 |
| ARG X HMB | | 0.637 | 0.060 | 0.101 | 0.115 |
| SEM (36)[3] | | 0.086 | 0.615 | 0.271 | 1.08 |

[1]All data represents the mean of 10 sample birds per treatment. [2]Glycogen status index =(mg total liver glycogen + mg muscle glycogen)/ g BW. [3]SEM (36) = pooled standard error of the mean with 36 degrees of freedom. [a,b]Means within a column with different superscripts are significantly different (P<.05).

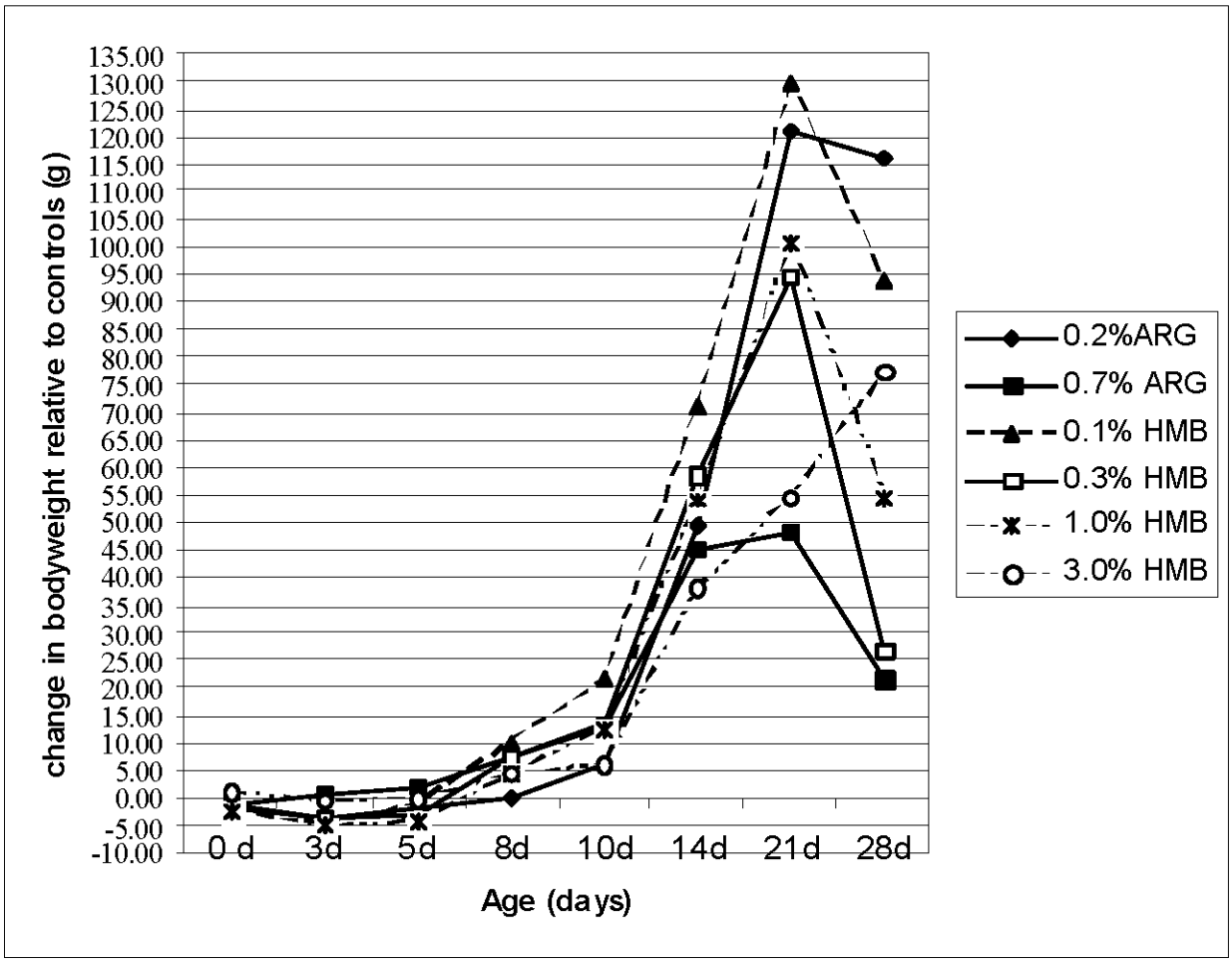


Fig. 1: The dose responsive effects of IOF[1] of arginine (ARG) and/or Beta-hydroxy- Beta-methylbutyrate (HMB) on the change in bodyweight relative to the non-injected controls from the day of hatch to 28 days post-hatch in turkeys[2].

[1]All *in ovo* feeding (IOF) solutions were prepared in 0.4% saline.

[2]All data represents the difference in the mean value of the bodyweights for each *in ovo* feeding (IOF) treatment and the mean value of the bodyweights for the non-injected controls with 20 sample birds per treatment.

Table 7: The effects of IOF[2] of arginine (ARG) and Beta-hydroxy-Beta-methylbutyrate (HMB) on hepatic glucose-6-phosphatase activity of turkeys at hatch and 7 days post-hatch.[1]

| IOF treatment | | Days of Age | |
|---|---|---|---|
| ARG | HMB | Day Hatch | Day 7 |
| | | (milli Units Glucose - 6 - phosphatase /mL/ug protein) | |
| 0 | 0 | 27.1[b] | 19.6[a] |
| 0 | 0.1% | 43.3[a] | 14.0[b] |
| 0.7% | 0 | 41.0[a] | 16.6[ab] |
| 0.7% | 0.1% | 43.4[a] | 19.4[a] |
| Source of Variation | | P-value | |
| ARG | | 0.02 | 0.42 |
| HMB | | 0.02 | 0.35 |
| ARG X HMB | | 0.03 | 0.01 |
| SEM (36)[2] | | 1.50 | 0.737 |

[1]All data represents the mean value of 10 sample birds per treatment. [2]SEM (36) = pooled standard error of the mean with 36 degrees of freedom. [a,b]Means within a column with different superscripts are significantly different (P<.05).

Consequently, the greater post-hatch energy reserves and circulating growth factors of the *in ovo* fed poults, likely enhanced post-hatch growth and may decrease early mortality.

All poults experienced a precipitous temporal decline in hepatic glycogen at 7 d post-hatch. Hepatic G6P activity had declined to half the activity observed at the day of hatch, suggesting that poults may acquire the majority of energy from consuming an external diet. This metabolic shift in glucose metabolism may be marked by a significant decline in hepatic glycogen. Consequently, at 14 d post-hatch, the poults *in ovo* fed HMB, ARG, HMB + ARG, and the controls had 70, 40, 19, and 32 times greater total hepatic glycogen levels than those poults sampled at 7 d post-hatch, respectively.

Muscle glycogen reserves of poults were not affected by *in ovo* feeding HMB with or without ARG, so we reject our initial hypothesis that these treatments would enhance muscle glycogen reserves. Glucose uptake by the muscles and tissues is mediated by the action of insulin (Langslow *et al.*, 1970), stored as glycogen and can be readily accessed for energy to fuel working muscles. Unlike liver glycogen, muscle glycogen is only available within the muscles and cannot be released into the circulation. The data from this experiment suggests that *in ovo* feeding of non-carbohydrate nutrients, such as HMB and ARG, may not elicit the release of insulin; thus, most of the glucose produced from hepatic gluconeogenesis is stored as glycogen in the liver and not available in the muscles.

Approximately 2 to 5% of all poults do not survive the first 14 days post-hatch due to limited energy reserves. Conventional hatchlings have limited energy reserves due to depletion of yolk stores, and a lag in the time for the bird to initiate feed intake. *In ovo* feeding addresses this issue by providing young birds with nutrients into the amnion of the developing embryo, and it is not dependent on the bird to initiate feed intake. Therefore, commercial application of *in ovo* feeding may greatly improve the survivability and hatchability of oviparous species.

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