The Effect of Early Nutrition on Satellite Cell Dynamics in the Young Turkey

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ABSTRACT Early posthatch satellite cell mitotic activity is an important aspect of muscle development. An understanding of the interplay between nutrition and satellite cell mitotic activity will lead to more efficient meat production. The objective of this study was to test the influence of the leucine metabolite, \( \beta \)-hydroxy \( \beta \)-methylbutyrate (HMB), and feed deprivation on muscle development in the early posthatch poult. Male Nicholas poultats were placed on 1 of 4 treatments: immediately fed a starter diet with 0.1% HMB (IF-HMB), immediately fed a starter diet containing 0.1% Solka-Floc for a control (IF-No HMB), feed and water withheld for 48 h immediately posthatch and then fed the HMB diet (WF-HMB), and feed and water withheld for 48 h immediately posthatch and then fed the control starter diet (WF-No HMB). 5-bromo-2'-deoxyuridine (BrdU) was injected intra-abdominaly into all poultats to label mitotically active satellite cells. The pectoralis thoracicus was harvested 2 h after the BrdU injection. Immunohistochemistry for BrdU, Pax7, and laminin along with computer-based image analysis was used to study muscle development. IF-HMB poultats had higher body weights (\( P < 0.01 \)) at 48 h and 1 wk of age and had higher satellite cell mitotic activity at 48 h of age (\( P < 0.01 \)) compared with the IF-No HMB and WF poultats. Therefore, dietary supplementation of HMB may have an anabolic effect on early posthatch muscle.

(Key words: \( \beta \)-hydroxy \( \beta \)-methylbutyrate, 5-bromo-2'-deoxyuridine, feed deprivation, Pax7)

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INTRODUCTION

Early posthatch nutrition (i.e., before 3 d of age) has an important impact on muscle development in the poult because the energy required to emerge from the shell leaves the newly hatched poult in a nutrient-deficient state (Uni and Ferket, 2004). Glycogen is the primary energy source available to the fetus when hatching; however, upon completion of the hatching process, the poult has greatly decreased its glycogen stores (John et al., 1987, 1988), thereby increasing the need for nutrients. Early nutrition is also important for the development of the gastrointestinal tract and the enzymes associated with digestion (Uni, 1998). The first week posthatch is also a time of major organ growth and development in the poult (Lilburn, 1998). An improvement in the development of the gastrointestinal tract and organ growth will allow for a more efficient uptake of nutrients for muscle development. Noy and Sklan (1999a) found that poultats offered nutrients immediately following hatch in a variety of forms exhibited a higher body weight and breast meat yield at market age. Also poultats fed immediately posthatch show a higher level of satellite cell mitotic activity in vitro compared with feed deprived animals (Halevy et al., 2003).

Satellite cells are located between the basal lamina and the sarcolemma of the myofiber (Mauro, 1961; Campion, 1984), and they are present in the avian embryo by mid-fetal stages of development (Feldman and Stockdale, 1992). Once the poult has hatched, adult satellite cells are present and myonuclei are postmitotic, eliminating the possibility of myonuclear accretion via mitosis of the existing myonuclei. However, myogenic satellite cells are mitotically active, and they are the source for new myonuclei in the posthatch muscle. A protein involved in myogenic satellite cell dynamics is Pax7, which is important for satellite cell specification because muscle deficient in Pax7 does not contain any satellite cells (Seale et al., 2000). Quiescent satellite cells express Pax7 (Zammit and Beauchamp, 2001; Asakura et al., 2002) but do not express the hematopoietic stem cell markers Sca-1 and CD45, suggesting that satellite cells are a distinct population of myogenic cells different from stem cells (Asakura et al., 2002). However, Pax7 expression is proposed to signal commitment of a cell to the myogenic lineage because myogenic stem cells developed into myoblasts expressing Pax7 following injury (Seale et al., 2004).

Satellite cell mitotic activity in the turkey is highest early posthatch and decreases with age as the turkey matures (Mozdziak et al., 1994). This suggests that the

Abbreviation Key: BrdU = 5-bromo-2'-deoxyuridine; FITC = fluorescein-isothiocyanate; HMB = \( \beta \)-hydroxy \( \beta \)-methylbutyrate; IF = immediately fed; IF-HMB = immediately fed a starter diet with 0.1% HMB; IF-No HMB = immediately fed a starter diet; PI = propidium iodide; WF = collectively feed deprived; WF-HMB = withheld feed and water for 48 h immediately posthatch and then fed the HMB diet; WF-No HMB = withheld feed and water for 48 h immediately posthatch and then fed the control starter diet.

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immediate posthatch period is the most important time to improve breast meat yield via myonuclear accretion because the major source of myofiber growth in a turkey greater than 9 wk of age occurs via an increase in cytoplasmic volume to myonucleus ratio referred to as DNA unit size (Mozdziak et al., 1994). However, poults are commonly held for 48 to 72 h without access to feed and water, which depresses satellite cell mitotic activity (Haley et al., 2003).

Chicks denied access to food and water for the first 48 h posthatch show smaller body and breast muscle weights at market age than chicks denied access to feed and water from d 2 to 4 posthatch or d 4 to 6 posthatch (Haley et al., 2000), suggesting the immediate posthatch period is the most important time for programming mature breast muscle size. Other studies have shown that delayed access to feed and water for the first 48 h posthatch decreases body weight and breast meat yield (Nir and Levanon, 1993; Noy and Sklan, 1999b; Vieira and Moran, 1999a,b). Similar findings with turkeys also show that early posthatch feed deprivation decreases growth (Pinchasov and Noy, 1993), possibly programming the muscle to be smaller via decreased satellite cell mitotic activity. Poults with delayed access to feed immediately posthatch exhibit lower satellite cell mitotic activity when compared with their fed counterparts (Mozdziak et al., 2002b; Haley et al., 2003), suggesting the importance of early nutrition on determining muscle growth potential via early satellite cell mitotic activity. Birds with delayed access to nutrition also exhibit smaller duodenal and jejunal crypts as well as fewer crypts per villus (Geyra et al., 2001), which would result in a lower digestive capacity of the small intestine and could negatively influence muscle development.

As a result of the recently identified nutritional influence on satellite cell mitotic activity over the first few days posthatch, early posthatch diets should be formulated to maximize satellite cell mitotic activity. Dietary supplementation with the leucine metabolite β-hydroxy β-methylbutyrate (HMB) may increase satellite cell mitotic activity. HMB is endogenously derived from α-ketoisocapraate and eventually forms HMG-coenzyme A to form cholesterol (Nissen and Abumrad, 1997). However, it has been found that if an exogenous source of HMB is introduced to the diet, it is used by the animal beyond what is produced endogenously (Van Koevering and Nissen, 1992). Leucine may have more important physiological roles than the formation of HMB, and it has been shown to decrease the amount of proteolysis in the muscle as well as decrease muscle damage during a period of stress to the muscle (Gallagher et al., 2000; Panton et al., 2000; Vukovich et al., 2001). The lack of feed and water available to the poult immediately following hatch and the energy required for the poult to emerge from the egg may challenge all muscles in the body. Addition of HMB to the starter diet may counteract the challenges placed on early posthatch muscle. The first objective of this experiment was to understand satellite cell dynamics in the early posthatch period, and the second objective was to examine the influence of HMB on satellite cell mitotic activity in the newly hatched poult.

**MATERIALS AND METHODS**

**Turkeys**

Two hundred seventy-two male Nicholas poults were obtained from a commercial hatchery immediately following hatch, and they were placed into battery cages2 at North Carolina State University. All procedures involving animals were approved by the North Carolina State University Institutional Animal Care and Use Committee. Two diets were used in the study. Both diets were based upon a standard corn and soybean meal basal diet (Table 1). The basal diet contained 0.1% HMB or 0.1% Solka-Floc3 as the control. Solka-Floc is a nonnutritional cellulose fiber feed additive. The poults were divided into 4 treatments: immediate access to feed and water fed the HMB diet (IF-HMB), immediate access to feed and water fed the control diet (IF-No HMB),

### TABLE 1. Basal diet composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>43.5</td>
</tr>
<tr>
<td>Soybean meal (48% CP)</td>
<td>40.9</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.22</td>
</tr>
<tr>
<td>Dicalcium phosphate (18.5% P)</td>
<td>3.38</td>
</tr>
<tr>
<td>Poultry fat</td>
<td>2.9</td>
</tr>
<tr>
<td>Poultry meal (60% CP)</td>
<td>5.0</td>
</tr>
<tr>
<td>in-Methionine</td>
<td>0.34</td>
</tr>
<tr>
<td>Lysine-HCL</td>
<td>0.36</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.27</td>
</tr>
<tr>
<td>Choline chloride (60%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Trace mineral premix1</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin premix2</td>
<td>0.2</td>
</tr>
<tr>
<td>Selenium2</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1Supplied the following per kilogram of feed: 120 mg of Zn as ZnSO4; 120 mg Mn as Mn SO4.H2O; 80 mg of Fe as Fe SO4.H2O; 10 mg of Cu as Cu SO4; 2.5 mg of I as Ca(IO3)2; 10 mg of Co as CoSO4.

2Supplied the following per kilogram of feed: vitamin A, 26,000 IU; cholecalciferol, 8,000 IU; vitamin E, 90 mg as α-tocopherol acetate; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 16 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 4 mg; biotin, 0.500 mg; vitamin B12, 0.08 mg; ethoxyquin, 200 mg.

3Selenium premix supplied the following per kilogram of feed: 0.2 mg of Se as NaSeO3.

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2Alternative Design Manufacturing and Supply Inc., Silon Springs, AR.

3Fiber Sales and Development Corp., Urbana, OH.
withheld from feed and water for 48 h immediately posthatch then placed on the HMB diet (WF-HMB), and withheld from feed and water for 48 h immediately posthatch then placed on the control diet (WF-No HMB). The birds in the IF group were provided feed and water within 4 h of hatching. Seventeen birds were housed per cage, and there were 4 different cages for each treatment (68 birds per treatment). At least one bird from each cage was randomly chosen for each sampling interval (see below). Conditions were closely monitored to ensure that there were similar environmental conditions (temperature) in each cage. Birds were weighed at placement, at 48 h posthatch, and 1 wk of age. Pectoralis thoracicus and pectoralis supracoracoideus muscles were weighed at 1 wk of age.

**Tissue Sampling**

After birds were randomly chosen from each treatment group, 5-bromo-2′-deoxyuridine (BrdU; 10 mg/mL; 10 mg BrdU/100 g of bird weight), a thymidine analog, was administered intra-abdominally using a 27-gauge hypodermic needle. After injection with BrdU, the pouls were maintained for 2 h before sampling to allow for the incorporation of the BrdU into the nuclei entering the S-phase of the cell cycle, when the birds were euthanized with Euthasol (0.25 mL/kg of BW). The left pectoralis thoracicus was harvested from poult at 24 h posthatch, 48 h posthatch, and 1 wk of age. The samples taken at 24 and 48 h posthatch included 5 pouls from each immediately fed (IF) treatment and 5 pouls from the collectively feed deprived group (WF). Five birds were sampled from all 4 groups at 1 wk posthatch. Immediately after the tissue was harvested, it was flash frozen in cooled isopentane. The tissues were then placed in cryovials and stored at −80°C until sectioning. The left pectoralis thoracicus was harvested from poult at 24 h posthatch, 48 h posthatch, and 1 wk of age. Pectoralis thoracicus and pectoralis supracoracoideus muscles were weighed at 1 wk of age.

**Immunohistochemistry**

Slides were brought to room temperature, and the sections were fixed with Carnoy’s solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) before staining for BrdU. All other sections were fixed with 4% paraformaldehyde in PBS (pH 7.4). The sections were washed 3 times for 5 min with PBS. Sections for BrdU and Pax7 staining were treated with 0.07 N NaOH for 5 min, and PBS was used to neutralize the NaOH. Three primary antibodies were added to the sections and incubated at 4°C in a humidified chamber overnight: monoclonal mouse anti-BrdU diluted 1:20 in PBS containing 0.5% Tween-20 and 0.5% bovine serum albumin, polyclonal rabbit anti-laminin diluted 1:30 in PBS containing 0.5% Tween-20 and 0.5% bovine serum albumin, and monoclonal mouse anti-Pax7-undiluted. Each primary antibody was incubated with a different serial section from each treatment group. After the tissue sections were blocked with PBS containing 10% goat serum and 0.5% Tween-20. Laminin was detected using goat anti-rabbit IgG conjugated to Texas red diluted 1:35 in PBS containing 10% goat serum and 0.5% Tween-20. Laminin was detected using goat anti-rabbit IgG conjugated to Texas red diluted 1:35 in PBS containing 10% goat serum and 0.5% Tween-20. Propidium iodide (PI; 50 g/mL in PBS) was added to all sections except for sections stained for laminin for 20 min to label all nuclei. Finally, the sections were placed in mounting media [75% glycerol (vol/vol), 25% dH2O (vol/vol), 75 mM KCL, 10 mM Tris, 2 mM MgCl2, 2 mM ethylene glycol-bis (β-amino-ethyl ether)-N,N,N’,N’-tetraacetatic acid, 1 mM NaNO3, pH 8.5, and 1 mg of P-phenylenediamine/mL], and a cover slip was sealed over the section using nail polish.

**Image Analysis**

A Leica DMR microscope with epiflorescence illumination was used to observe the tissue sections. All nuclei and laminin were observed with PI filter set, and the BrdU- and Pax7-labeled nuclei were observed with a FITC filter set. A Spot-RT CCD camera was used to capture the images of nuclei visualized by the FITC and PI filter sets. The FITC- and PI-labeled nuclei were counted using Image-Pro Plus software. The number of BrdU-labeled nuclei per 1,000 PI-labeled nuclei was used as an index for satellite cell mitotic activity. The Pax7 labeling index was determined by the number of Pax7-positive nuclei per 1,000 PI-labeled nuclei. The criteria for completing nuclear analysis was counting at least 1,000 PI-labeled nuclei. The Pax7 to BrdU ratio was determined by dividing the Pax7 labeling index by the BrdU labeling index. The area of muscle section containing the count of PI-labeled nuclei was also determined. The number of PI-labeled nuclei was expressed relative to the section area that was analyzed. Laminin staining was used to demarcate myofiber borders. Image-Pro Plus software was also used to determine myofiber cross-sectional area for at least 100 myofibers per muscle sample.

**Statistics**

The general linear models procedure of SAS (SAS Institute, 1985) was used to perform a one-way analysis.
of variance to determine the treatment effect on 48 h posthatch weight, 1 wk weight, body weight gain, muscle weights, muscle weight to body weight ratio, the number of PI-labeled nuclei per muscle area, myofiber cross-sectional area, satellite cell mitotic activity, Pax7 labeling index, and Pax7/BrdU ratio. Means were separated using least significant differences (Zar, 1999). A 2 × 2 factorial analysis was performed on the 1 wk data. Statistical significance was accepted at P < 0.05.

RESULTS

Growth

The body weights of the IF-HMB group were significantly higher than those of the other 3 groups at 48 h posthatch (Table 2). Both of the WF groups at 48 h posthatch weighed significantly (P < 0.01) than either of the IF groups. At 1 wk of age, the pooled HMB fed birds had significantly higher body weights than the control diet fed poult (Table 3). Likewise, the pooled IF birds significantly outweighed the WF birds at 1 wk posthatch. Also the IF-No HMB poult weighed significantly less than the IF-HMB birds. The WF-HMB birds were also significantly heavier than the WF-No HMB birds. The amount of gain at 1 wk of age was higher in the pooled HMB groups than for the pooled poult on the control diet, and gain was also higher in the IF group than the WF group. The gain for the IF-HMB birds was higher than the IF-No HMB group; similarly, the WF-HMB group was higher than the WF-No HMB group.

At 1 wk of age, pectoralis thoracicus weights were significantly higher in the IF-HMB group compared with the IF-No HMB, IF, and WF groups and the WF-HMB and WF-No HMB groups (Table 4). However, there were no differences in the ratio of pectoralis thoracicus and pectoralis supracoracoideus to body weight except between the IF and WF groups. The only differences in 1 wk pectoralis supracoracoideus weights were between the HMB pooled poult and the control fed poult; and between the IF and WF groups. Myofiber cross-sectional areas were smaller in the WF group at 24 h posthatch, and a difference between all groups existed at 48 h posthatch with the IF-HMB group achieving the largest cross-sectional area (Table 5). At 1 wk of age, the HMB group had a larger cross-sectional area than the control fed group, and the IF group had a larger cross-sectional area than the WF group (Table 6). IF-HMB poult had larger cross-sectional area than the IF-No HMB birds. There was no difference in myofiber cross-sectional area between the WF-HMB poult and the IF-No HMB poult.

Satellite Cell Mitotic Activity

At 24 and 48 h posthatch, the IF-HMB poult had a higher index of satellite cell mitotic activity than the IF-No HMB and WF poult (Table 5). However, the IF-No HMB BrdU labeling index was higher compared with the WF at 48 h. Interestingly, the WF pooled group had a higher BrdU labeling index than the IF pooled poults at 1 wk of age (Table 6), indicating a level of compensation following feed deprivation. However, there was no difference at 1 wk of age between the pooled diet treatments or the comparison of IF-HMB to IF-No HMB and WF-HMB to WF-No HMB.

At 24 h posthatch, there were no differences in Pax7 labeling index; however, at 48 h posthatch the WF group had a higher level of Pax7 labeling index by the BrdU labeling index. There were no differences at 1 wk of age (Table 6). A ratio of Pax7 to BrdU labeling index was calculated to determine the number of satellite cells in the proliferative reserve (Haley et al., 2004; Oustanina et al., 2004) compared with the number of cycling satellite cells by dividing the Pax7 labeling index by the BrdU labeling index. There were no differences in the Pax7/BrdU ratio at 24 h posthatch (Table 5). However, at 48 h posthatch the WF group had the highest ratio, possibly indicating a shutdown of cycling satellite cells and an increase in the number of satellite cells in the proliferative reserve awaiting activation upon feeding. There were no differ-
TABLE 4. Pectoralis thoracicus weight, pectoralis supracoracoideus weight, pectoralis thoracicus per body weight ratio, and pectoralis supracoracoideus per body weight ratio at 1 wk of age

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pectoralis thoracicus</th>
<th>Pectoralis thoracicus ratio</th>
<th>Pectoralis supracoracoideus</th>
<th>Pectoralis supracoracoideus ratio</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMB pooled²</td>
<td>3.24a</td>
<td>0.022a</td>
<td>0.75a</td>
<td>0.005a</td>
<td>10</td>
</tr>
<tr>
<td>No-HMB pooled²</td>
<td>2.63b</td>
<td>0.021b</td>
<td>0.61b</td>
<td>0.005b</td>
<td>10</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.16</td>
<td>0.001</td>
<td>0.04</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>IF pooled³</td>
<td>4.01a</td>
<td>0.026a</td>
<td>0.94a</td>
<td>0.006a</td>
<td>10</td>
</tr>
<tr>
<td>WF pooled³</td>
<td>1.87b</td>
<td>0.017b</td>
<td>0.43b</td>
<td>0.004b</td>
<td>10</td>
</tr>
<tr>
<td>Pooled SE</td>
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<td>0.001</td>
<td>0.04</td>
<td>0.001</td>
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</tr>
<tr>
<td>IF-HMB</td>
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<td>0.027a</td>
<td>1.01a</td>
<td>0.006a</td>
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<tr>
<td>IF-No HMB</td>
<td>3.61a</td>
<td>0.025a</td>
<td>0.86a</td>
<td>0.006a</td>
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<td>Pooled SE</td>
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<td>0.001</td>
<td>0.69</td>
<td>0.001</td>
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<tr>
<td>WF-HMB</td>
<td>2.07a</td>
<td>0.017a</td>
<td>0.49a</td>
<td>0.004a</td>
<td>5</td>
</tr>
<tr>
<td>WF-No HMB</td>
<td>1.66b</td>
<td>0.016b</td>
<td>0.37a</td>
<td>0.003b</td>
<td>5</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.11</td>
<td>0.001</td>
<td>0.041</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values within columns of the same pairing without a common superscript are significantly different (P < 0.05).

1IF-HMB = immediately fed a starter diet with 0.1% HMB; IF-No HMB = immediately fed a starter diet; WF-HMB = witheld feed and water for 48 h immediately posthatch and then fed the HMB diet; WF-No HMB = withheld feed and water for 48 h immediately posthatch and then fed the control starter diet.

2 HMB pooled = overall mean HMB treated birds at 1 wk of age; No-HMB pooled = overall mean non-HMB treated birds at 1 wk of age.

3IF pooled = overall mean fed birds at 1 wk of age; WF pooled = overall mean previously feed deprived birds at 1 wk of age.

ences at 1 wk of age, except the IF group had a higher ratio than the WF group (Table 6).

The number of PI-labeled nuclei per area measured was not different at 24 h posthatch between any groups. However, the PI per area measured at 48 h posthatch was higher in the IF-No HMB birds than the IF-HMB (Table 5), suggesting a larger DNA unit size in the HMB fed birds. At 1 wk of age the pooled HMB group had a lower PI per area measured than the pooled control fed group. Both HMB fed groups had lower PI per area measured than their control fed counterparts (Table 6).

**DISCUSSION**

The HMB is a metabolite of leucine and may be involved in the increase in cholesterol synthesis for membrane protection after it is metabolized to HMG-coenzyme A (Nissen and Abumrad, 1997). After the injection of HMB in lambs and pigs, only 34% was recovered in the urine, indicating use of the metabolite by animals (Van Koevering and Nissen, 1992). Other studies have concluded that the administration of HMB decreases proteolysis while it increases fat-free mass (Gallagher et al., 2000; Panton et al., 2000; Vukovich et al., 2001), which may be beneficial to animal agriculture.

In vitro, chicken muscle shows a decrease in proteolysis after the administration of HMB (Ostaszewski, 2000). However, HMB does not seem to be as effective in more mature animal muscle because broiler chickens showed no increase in body weight and carcass yield at 42 d following HMB supplementation, and weanling pigs did not show an increase in average daily gain following

**TABLE 5. Satellite cell mitotic activity, Pax7 labeling index, Pax7/5-bromo-2'-deoxyuridine (BrdU) labeling ratio, myofiber cross-sectional area (CSA), nuclei per unit area by treatment at 24 and 48 h of age**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IF-HMB</td>
<td>IF-No HMB</td>
</tr>
<tr>
<td>Satellite cell mitotic activity²</td>
<td>0.075a</td>
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</tr>
<tr>
<td>Pax7 labeling index³</td>
<td>0.096a</td>
<td>0.13a</td>
</tr>
<tr>
<td>Pax7/BrdU ratio³</td>
<td>1.28b</td>
<td>3.52b</td>
</tr>
<tr>
<td>Myofiber CSA⁴ (µm²)</td>
<td>19a</td>
<td>17a</td>
</tr>
<tr>
<td>Nuclei² (n/µm²)</td>
<td>0.0167a</td>
<td>0.0207a</td>
</tr>
<tr>
<td>Sample size</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Values within rows of the same age group without a common superscript are significantly different (P < 0.05).

1IF-HMB = immediately fed a starter diet with 0.1% HMB; IF-No HMB = immediately fed a starter diet; WF = withheld feed and water for 48 h immediately posthatch.

2Satellite cell mitotic activity is expressed as the number of BrdU-fluorescein isothiocyanate-labeled nuclei per 1,000 propidium iodide-labeled nuclei.

3Pax7 labeling index is expressed as the number of Pax7-fluorescein isothiocyanate-labeled nuclei per 1,000 propidium iodide-labeled nuclei.

4Pax7/BrdU ratio is the Pax7 labeling index/BrdU labeling index.

5CSA = cross-sectional area.

6Number of propidium iodide-labeled nuclei per square micrometer of tissue section.
TABLE 6. Satellite cell mitotic activity, Pax7 labeling index, Pax7/BrdU labeling ratio, myofiber cross-sectional area (CSA), nuclei per unit area by treatment at 1 wk of age

<table>
<thead>
<tr>
<th>Item</th>
<th>HMB No-HMB</th>
<th>Pooled IF</th>
<th>Pooled WF</th>
<th>IF- No Pooled</th>
<th>WF-No Pooled</th>
<th>HMB pooled</th>
<th>No-HMB pooled</th>
<th>SE</th>
<th>IF pooled</th>
<th>SE</th>
<th>WF pooled</th>
<th>SE</th>
<th>Pooled IF- SE</th>
<th>Pooled WF- SE</th>
<th>HMB 3</th>
<th>SE</th>
<th>HMB 3</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satellite cell mitotic activity</td>
<td>0.069a</td>
<td>0.095a</td>
<td>0.011</td>
<td>0.063 b</td>
<td>0.10a</td>
<td>0.0016</td>
<td>0.055 a</td>
<td>0.071a</td>
<td>0.008</td>
<td>0.0038</td>
<td>0.066a</td>
<td>0.003</td>
<td>0.055 a</td>
<td>0.003</td>
<td>0.083 a</td>
<td>0.022</td>
<td></td>
<td></td>
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<tr>
<td>Pax7 labeling index</td>
<td>0.067a</td>
<td>0.069a</td>
<td>0.004</td>
<td>0.070 a</td>
<td>0.066a</td>
<td>0.0038</td>
<td>0.068 a</td>
<td>0.073a</td>
<td>0.004</td>
<td>0.0038</td>
<td>0.069a</td>
<td>0.003</td>
<td>0.066a</td>
<td>0.003</td>
<td>0.066 a</td>
<td>0.0062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pax7/BrdU ratio</td>
<td>1.03a</td>
<td>0.73a</td>
<td>0.12</td>
<td>1.12 a</td>
<td>0.67b</td>
<td>0.12</td>
<td>1.23 a</td>
<td>1.02a</td>
<td>0.17</td>
<td>0.78 a</td>
<td>0.56a</td>
<td>0.19</td>
<td>0.78 a</td>
<td>0.150</td>
<td>0.78 a</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myofiber CSA (µm²)</td>
<td>114 a</td>
<td>63b</td>
<td>12</td>
<td>121 a</td>
<td>56b</td>
<td>12</td>
<td>156 a</td>
<td>87b</td>
<td>20</td>
<td>73 a</td>
<td>39a</td>
<td>13</td>
<td>73 a</td>
<td>13</td>
<td>73 a</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclei (n/µm²)</td>
<td>0.0090 b</td>
<td>0.0106a</td>
<td>0.003</td>
<td>0.0077 b</td>
<td>0.0119a</td>
<td>0.006</td>
<td>0.00670 b</td>
<td>0.00868a</td>
<td>0.006</td>
<td>0.0113 b</td>
<td>0.0126a</td>
<td>0.001</td>
<td>0.0113 b</td>
<td>0.006</td>
<td>0.0113 b</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample size: 10

a,bValues within rows of the same pairing without a common superscript are significantly different (P < 0.05).

HMB pooled = mean over all HMB treated birds at 1 wk of age; No-HMB pooled = mean over all non-HMB treated birds at 1 wk of age.

IF pooled = mean over all fed birds at 1 wk of age; WF pooled = mean over all previously feed deprived birds at 1 wk of age.

IF-HMB = immediately fed a starter diet with 0.1% HMB; IF-No HMB = immediately fed a starter diet; WF-HMB = withheld feed and water for 48 h immediately posthatch and then fed the HMB diet; WF-No HMB = withheld feed and water for 48 h immediately posthatch and then fed the control starter diet.

Satellite cell mitotic activity is expressed as the number of BrdU-fluorescein isothiocyanate-labeled nuclei per 1,000 propidium iodide-labeled nuclei.

Pax7 labeling index is expressed as the number of Pax7-fluorescein isothiocyanate-labeled nuclei per 1,000 propidium iodide-labeled nuclei.

Pax7/BrdU ratio is the Pax7 labeling index/BrdU labeling index.

CSA = cross-sectional area.

HMB supplementation (Nissen et al., 1994; Gatnau et al., 1995). Our results indicate that HMB may be more effective in young muscle, but it is unclear if the influence will be maintained throughout production. The effectiveness of HMB in young muscle may be due to the extreme growth potential and the high level of satellite cell activity of the young animal. Broiler chickens only showed a short-lived improvement in growth administration of 0.1% HMB (Nissen et al., 1994). The ability of young muscle to program ultimate size via satellite cell mitotic activity indicates that the best time to feed a muscle promont is early in life. Because satellite cell mitotic activity decreases with age (Mozdziak et al., 1994) feeding HMB in the more mature animal may be ineffective if the intention is to increase muscle size at market age through a satellite cell pathway. Also, by programming an increase in muscle size early posthatch in the turkey, diets may need to be reformulated to account for the increased potential to show increase in muscle performance.

The HMB has also been shown to decrease proteolysis following a period of stress in the muscle by decreasing the proteolytic enzyme calpain (Jank et al., 2000). However, in chicks, calpain transcriptional activity was not influenced by feed deprivation or nutrient level (Mozdziak et al., 2002a), suggesting muscle dynamics early posthatch influenced by HMB may be the result of another mechanism. Also, the increased level of BrdU labeling in the IF-HMB pouls indicates that HMB may be acting through a mechanism other than decreased proteolysis. The increased level of satellite cell mitotic activity in the IF-HMB pouls, as well as an increase in myofiber cross-sectional area, may be the result of HMB increasing the production of growth factors such as insulin-like growth factor and fibroblast growth factor, which have been shown to increase satellite cell proliferation and protein synthesis in turkey satellite cells (Dodson et al., 1996; McFarland, 1999) and may result in increased satellite cell mitotic activity and myofiber cross-sectional area. HMB may also increase insulin production, which has been shown to increase DNA synthesis (i.e., satellite cell activity) and overall cell size (Bikopoulos et al., 2004).

It is unclear if HMB will result in an increase in meat yield at market age of poults following a period of feed deprivation. However, HMB improved gain and overall body weight in WF-HMB pouls when compared with WF-No HMB treatment birds. Feed deprivation decreases satellite cell mitotic activity early in life, which leads to a decrease in overall skeletal muscle growth and meat yield at market age (Halevy et al., 2000). It is detrimental to overall meat production to decrease satellite cell mitotic activity early in life because it is the time with the highest level of DNA accretion in the poult. Further indicating the importance of satellite cell mitotic activity early in life, a decrease in activity will result in a reduction in muscle size at maturity (Mozdziak et al., 1997, 2000). Myonuclei are not mitotically active posthatch. Therefore, the myofibers must rely on...
myogenic satellite cells to fuel myonuclear accretion. Because myofibers are multinucleate, the more nuclei a muscle can acquire early in life, the larger the potential for muscle size at market age because DNA unit number governs muscle size (Mozdziak et al., 1994). A factor governing muscle growth in the turkey is a result of protein synthesis; however, the more nuclei within the muscle, the more protein synthesis that can occur. Turkeys show a decrease in satellite cell mitotic activity between 3 to 9 wk of age with virtually no growth occurring via satellite cell mitotic activity from 9 to 26 wk of age (Mozdziak et al., 1994), indicating the importance of early posthatch satellite cell activity.

Feed deprivation caused a decrease in satellite cell mitotic activity at 48 h posthatch in the WF poult when compared with fed pouls in this study. Similar findings have been reported in other research (Mozdziak et al., 2002b; Halevy et al., 2003), suggesting a missed opportunity for muscle development. Interestingly, feed-deprived pouls exhibited a higher level of satellite cell mitotic activity at 1 wk of age supporting the work of Halevy et al. (2003), who reported a higher level of satellite cell mitotic activity after a period of feed deprivation when compared with fed pouls at 6 d of age. However, Mozdziak et al. (2002b) did not find any compensatory satellite cell mitotic activity following the period of feed deprivation. The difference in results between Mozdziak et al. (2002b) and the current study could be due to differences in methodology. The present experiment used a single pulse injection of BrdU showing satellite cell mitotic activity at that particular time; whereas Mozdziak et al. (2002b) used a continual infusion of BrdU via an implanted pump providing a continuous readout of satellite cell mitotic activity over 1 wk, which could have masked the compensatory response. The satellite cell population may be attempting to compensate for the missed development window for myonuclear accretion. However, it is unlikely that this increase in satellite cell mitotic activity would result in a complete recovery of the muscle because the critical period of satellite cell mitotic activity immediately posthatch was missed.

Chicks that have been fed after a period of feed deprivation show a lower body weight and breast meat yield at market age when compared with chicks fed immediately (Nir and Levanon, 1993; Halevy et al., 2000). Muscle, following inactivity, normally shows an increase in satellite cell mitotic activity (Mozdziak et al., 1997; Mozdziak et al., 2000). However, an increase in satellite cell mitotic activity after a period of inactivity is short lived and does not completely compensate for the previous absence of satellite cells (Mozdziak et al., 1997, 2000).

Another indicator of muscle development is Pax7, which is a paired box transcription factor that is essential for the development and specification of satellite cells (Seale et al., 2000; Asakura et al., 2002). Once a cell expresses Pax7, it becomes committed to the myogenic lineage (Zammit and Beauchamp, 2001). In the current study, Pax7 labeling index was higher in the WF pouls 48 h after hatch compared with pouls with immediate access to feed and water. The same group of pouls had a low level of satellite cell mitotic activity, indicating that when there is a depressed level of satellite cell mitotic activity, there is a relative increase in the number of quiescent cells committed to the myogenic lineage preparing for an expected time of compensatory activity. Current research (Seale et al., 2004) has shown that uncommitted cells isolated from uninjured muscle do not commit to the myogenic lineage and do not express Pax7, but in injured muscle uncommitted cells are recruited to the myogenic lineage and express Pax7. It is possible that satellite cells expressing Pax7 represent a subpopulation of satellite cells that holds the proliferative reserve of the overall satellite cell population (Halevy et al., 2004). The increased level of Pax7 expression and decreased level of satellite cell mitotic activity in the WF pouls compared with the IF pouls at 48 h of age shows a relative increase in the satellite cell proliferative reserve. The compensatory increase in satellite cell mitotic activity in the WF pouls compared with the IF pouls at 1 wk of age without a difference in Pax7 expression supports the notion that the proliferative reserve was being conserved at 48 h in the WF pouls.

Contradictory to past findings (Seale et al., 2000), recent research has shown that mice lacking Pax7 may contain a small number of satellite cells from embryonic formation, but these mice have fewer satellite cells at maturity than wild-type mice (Oustanina et al., 2004), suggesting Pax7 is necessary for the replenishment of cycling satellite cells. Also, a recent study has shown that the expression of Pax7 in a majority of satellite cells is downregulated during proliferation (Zammit et al., 2004). However, a small population of satellite cells expresses Pax7 during proliferation and, subsequently, does not differentiate but leave the cell cycle and once again become quiescent satellite cells (Zammit et al., 2004). This indicates that Pax7 expression marks the proliferative reserve for satellite cell renewal, and it also supports the concept of satellite cell heterogeneity (Schultz, 1996).

The HMB may improve muscle development via an increase in myogenic satellite cell mitotic activity in the immediate posthatch turkey. Feed deprivation also detrimentally influences muscle development in the turkey. Other studies support the findings that nutrition can influence myogenic satellite mitotic activity immediately posthatch (Mozdziak et al., 2002b). However, the exact anabolic mechanism for HMB to promote satellite cell mitotic activity is unknown. Further research may focus on understanding the relationship of myogenic stem cells and myogenic satellite cells regarding myonuclear accretion in the young animal. The area of myonuclear accretion, satellite cells, and myogenic stem cells is a relatively unexplored area in animal agriculture and may provide an effective and promising means of increasing breast meat production in the turkey.
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