Satellite Cell Mitotic Activity of Broilers Fed Differing Levels of Lysine

Simone Pophal¹, Paul E. Mozdzian² and Sérgio L. Vieira²
¹Departamento de Zootecnia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
²Department of Poultry Science, North Carolina State University, Raleigh, North Carolina, USA
E-mail: pemozdzi@unity.ncsu.edu

Abstract: Post-hatch myofiber growth is dependent upon the addition of new nuclei from the mitotically active satellite cell population. The objective of this study was to examine the relationship between different levels of dietary lysine and satellite cell mitotic activity during the early post-hatch period. Broiler chicks were split into five groups of 10 birds each immediately post-hatch. One group was not provided any feed or water for the first three days post-hatch, whereas the other groups were provided a standard starter diet with different levels of lysine (0.82, 0.98, 1.16, 1.33%) for the first three days post-hatch. All birds were injected with 5-Bromo-2-deoxyuridine (BrDU) 2 hours before they were killed on the third day post-hatch. Mitotically active satellite cells were identified in the Pectoralis thoracicus and quantified using BrDU immunohistochemistry in combination with computer-based image analysis. Satellite cell mitotic activity was significantly (P < 0.05) lower in the starved compared to any of the fed groups. However, satellite cell mitotic activity was highest (P < 0.05) in the birds that were provided a lysine deficient diet (0.82%). The current study suggests that it is possible to nutritionally stimulate the satellite cell population in the early post-hatch chick, and that it is an important endeavour to re-examine the nutritional requirements of the early post-hatch chick to optimize meat yield.

Key words: Chicken, nutrition, muscle, myofiber, growth

Introduction
Post-hatch muscle growth occurs exclusively through an increase in myofiber size without an increase in myofiber number (Remignon et al., 1995). Concurrent with the increase in myofiber size is an increase in myofiber DNA content. However, the increase in myofiber DNA does not occur through pre-existing myonuclei because they are post-mitotic (Stockdale and Holtzer, 1961). The increase in myofiber DNA content occurs through the action of the mitotically active satellite cell population that is located between the sarcolemma and the myofiber basal lamina (Mauro, 1961; Yablonka-Reuveni, 1995; McFarland, 1999; Goldring et al., 2002; Morgan and Partridge, 2003). The role of the satellite cell population during normal skeletal muscle growth is to proliferate then donate nuclei to the growing myofiber. Satellite cell fusion with the myofiber provides the genetic machinery for the age-related increases in myofiber size. Previous research has indicated that postnatal myofiber growth in avian species can be considered to occur in at least two phases (Mozdzian et al., 1994). The first phase of myofiber growth occurs early in life, and it is characterized by a high level of satellite cell mitotic activity. However, later in life, satellite cell mitotic activity falls to low levels, and myofiber growth occurs almost exclusively through an increase in the volume of cytoplasm surrounding each nucleus (DNA Unit Size; Mozdzian et al., 1994; Allen et al., 1999; Mozdzian et al., 2000). Therefore, satellite cell mitotic activity early in life governs the ability of the muscle to meet its full potential genetic size. Furthermore, it has been shown through hind-limb unloading of juvenile muscle followed by reloading that a temporary suspension in satellite cell mitotic activity results in a reduction in mature muscle size under normal growth conditions (Mozdzian et al., 2000). Similarly, it has been shown that temporarily reducing satellite cell activity through irradiation of the turkey Pectoralis thoracicus results in a decrease in mature muscle size (Mozdzian et al., 1997). Therefore, inhibition of myonuclear accretion/myonuclear number results in a reduction in mature muscle size. The interval between hatching to placement on feed for chicks and turkey pouls may be as large as 72 hours. Delayed placement on feed results in a reduction in satellite cell activity (Halevy et al., 2000; Halevy et al., 2003; Mozdzian et al., 2002b), myonuclear apoptosis (Mozdzian et al., 2002a; Pophal et al., 2003), and most importantly a reduction in meat yield at market age (Vieira and Moran, 1999; Halevy et al., 2000). Therefore, the impact of nutrition on satellite cell mitotic activity during the early post-hatch period is very important in determining ultimate meat yield. Dietary lysine is also very important in poultry diets because it is an essential amino acid, it is associated with protein accretion, and growth has been correlated with the lysine content of poultry diets (Waldroup et al., 1976; Baker and Han, 1984). However, the relationship between dietary lysine levels and the mechanisms governing skeletal muscle growth have not been fully explored. The objective of this study was to examine the effect of dietary lysine level on...
satellite cell mitotic activity in the *Pectoralis thoracicus*. The rationale for the study was to understand the effect of dietary lysine levels on satellite cell mitotic activity, muscle growth potential, and muscle growth in the early post-hatch chick.

**Materials and Methods**

**Birds:** Fifty male broiler chicks (Cobb x Cobb 500) were selected by weight at hatch and groups (n=10) were assembled with the same (P > 0.05) mean weight (Table 2). Subsequently, the groups were randomly assigned to one of 5 different dietary treatments. The composition of all diets is shown in Table 1. The first group was not provided any food or water over the first three days post-hatch. All other groups were provided water ad libitum. The second group was provided with a lysine-deficient (low total protein) diet containing 0.82% digestible lysine. The third group was provided a lysine deficient diet containing 0.99% digestible lysine. The fourth group was provided an adequate level of digestible lysine 1.16%, and the last group was provided an elevated lysine level (1.33%; National Research Council, 1994). The experiment lasted from hatch until 3 days of age.

**BrdU Labeling:** At 3 days of age, birds were weighed and each chick was injected with 100 micrograms/mL BW 5-Bromo-2-deoxyuridine (BrdU). The birds were allowed to survive 2 hours after BrdU injection when they were killed by an overdose of Euthanol (Delmarva Laboratories, Midlothian, VA; 0.25mL/kg body). Immediately after death, the *Pectoralis thoracicus* was harvested from each bird, tied to wooden sticks at resting length, and immersed in Carnoy's solution (60% Ethanol, 30% Methanol, 10% Glacial Acetic Acid). Muscle samples were dehydrated, cleared, and embedded in paraffin. Subsequently, 8-micron thick sections were cut on a microtome, adhered to glass slides, cleared, hydrated, and immersed in 0.07 N NaOH for three minutes to denature the DNA. Subsequently, the base was neutralized with PBS, and the sections were incubated with an anti-BrdU monoclonal antibody (Becton Dickinson, Mountain View, CA) that was diluted 1:20 with PBS+10% Goat Serum+0.5% Tween-20. The primary antibody was detected with goat-anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC; ICN Biomedicals Inc, Irvine, CA). All nuclei in the sections were counter stained with propidium iodide (50 μg per mL/PBS). Sections were placed in mounting medium (75% (vol/vol) glycerol, 75 mM KCl, 10 mM tri(hydroxymethyl)aminomethane, 2 mM MgCl₂, 2 mM ethylene glycol-bis (β-aminooethyl ether)-N,N,N',N'-tetraacetic acid, 1 mM NaN₃, pH 8.5, 1 mg/mL phenylenediamine), and a cover slip was adhered to the glass slide with nail polish.

**Image Analysis:** Muscle sections were observed with a Leica DMR microscope (Leica Microsystems, Bannockburn IL) equipped with epifluorescence illumination. BrdU labeled nuclei were visualized with a fluorescein isothiocyanate filter set (Omega Optical, Brattleboro VT), and all nuclei were visualized with a propidium iodide filter set (Omega Optical, Brattleboro VT). The excitation and emission characteristics of fluorescein and propidium iodide are sufficiently different that each may be individually visualized with different filter sets. Images of nuclei visualized with the fluorescein isothiocyanate filters and nuclei visualized with the propidium iodide filters were acquired using a Spot-RT CCD camera (Diagnostic Instruments, Sterling Heights, MI). The number of fluorescein and propidium iodide labeled nuclei was determined using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). An index of satellite cell mitotic activity was expressed as the number of BrdU labeled nuclei per 100 propidium iodide labeled nuclei. The criteria for concluding nuclear analysis was counting at least 1,000 propidium iodide labeled nuclei. Myofiber cross-sectional areas were also determined using Image-Pro Plus software. The criteria for concluding myofiber cross-sectional area analysis for each muscle was determining myofiber cross-sectional area for at least 200 myofibers.

**Statistical Analysis:** Body weight, weight gain, feed intake, feed conversion, muscle weight, myofiber cross-sectional area, and satellite cell mitotic activity were analyzed using the General Linear Models procedure of SAS (SAS Institute, 1985) to perform a one-way-analysis of variance to determine the effect of diet on each parameter. In all cases, means were separated using least significant differences (Ott, 1993). If variances were unequal than a logarithmic transformation was performed on the data.

**Results**

Body weights were not different between treatment groups (P > 0.05) on the day of hatch. However, feed deprivation resulted in a lower (P < 0.05) body weight at 3 days of age (Table 2). Myofiber cross-sectional area was also lower (P < 0.05) in the birds that were not provided feed over the first three days of life compared to fed birds. However, there were no statistically significant differences (P > 0.05) in body weight, weight gain, feed conversion, muscle weight, or myofiber cross-sectional area between any of the treatments where feed was provided to the birds (Table 2). Satellite cell mitotic activity was lowest (P < 0.05) in the feed deprived chicks. Satellite cell mitotic activity was highest (P < 0.05) for birds fed the lysine deficient diet (0.82%) compared to both feed deprived birds and birds fed 0.99%, 1.16% or 1.33% lysine (Table 3).
## Table 1: Composition of Experimental Diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>True Fecal Digestible Lysine, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.82</td>
</tr>
<tr>
<td>Corn</td>
<td>66.50</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>27.00</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>2.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.85</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.35</td>
</tr>
<tr>
<td>Salt</td>
<td>0.40</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>0.56</td>
</tr>
<tr>
<td>Mineral premix(^a)</td>
<td>0.08</td>
</tr>
<tr>
<td>Choline-HCl</td>
<td>0.07</td>
</tr>
<tr>
<td>Vitamin premix(^a)</td>
<td>0.10</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.052</td>
</tr>
<tr>
<td>L-Lysine-HCl</td>
<td>0.040</td>
</tr>
<tr>
<td>L-threonine</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Nutrients (%)
- ME (kcal/kg): 2.950
- Crude Protein: 19
- Calcium: 0.95
- Av. P: 0.44
- Sodium: 0.30
- Potassium: 0.69
- Chlorine: 0.30
- Dg. Arginine: 1.02
- Dg. Lysine: 0.82
- Dg. Met+Cys: 0.59
- Dg. Tryptophan: 0.17
- Dg. Threonine: 0.59
- Dg. Valine: 0.78
- Dg. Leucine: 1.58
- Dg. Isoleucine: 0.68
- Dg. Histidine: 0.43
- Dg. Phenylalanine: 0.85
- Choline (mg/kg): 1500

\(^{a}\) Supplemented per kg: vitamin A, 5,000 IU; riboflavin, 500 IU; vitamin E, 15 IU; vitamin K, 2 mg; vitamin B\(_{12}\).
15 mg biotin, 0.15 mg folate, 1 mg niacin, 50 mg pantothenic acid, 25 mg pyridoxine, 5 mg riboflavin, 5 mg thiamin, 3 mg copper, 8 mg iodine, 0.5 mg iron, 100 mg manganese, 80 mg selenium, 0.15 mg zinc, 70 mg.

### Discussion

Given the recent studies about the effect of early nutrition on skeletal muscle growth (Halevy et al., 2000; Mozdziarz et al., 2002a, b; Halevy et al., 2003), it is not surprising that satellite cell mitotic activity and muscle size was lower (P < 0.05) in the birds that were not provided feed compared to birds that were provided feed immediately after hatch. It is also not surprising, given the short time-frame of this study, that there were no differences (P > 0.05) in body weight or myofiber diameter between the fed groups because the assays likely do not have enough sensitivity to reveal differences after only a three day feeding regimen. However, the satellite cell results are important because nutritionally induced decreases in satellite cell mitotic activity during the early phase of growth have been linked to decreases in meat yield at market age (Halevy et al., 2000). The most important finding from this study is that satellite cell mitotic activity early in life was elevated by a lysine deficient diet (Table 3). It has recently been demonstrated that satellite cells are a heterogeneous multi potential cell population (Seale and Rudnicki, 2000; Asakura et al., 2001; Hawke and Garry, 2001; White and Grounds, 2003), but the full functional significance of the multi potential cells has not yet been demonstrated for normal growing skeletal muscle. Most importantly, the majority of BrdU-labeled satellite cells fuse with the myofiber shortly after division (Moss and Leblond, 1971) showing that the present measure of satellite cell mitotic activity is an appropriate measure of
Table 2: Live performance of broilers fed increased lysine in an ideally balanced feed from 1 to 3 days of age

<table>
<thead>
<tr>
<th>Diets*</th>
<th>Starved</th>
<th>0.82%</th>
<th>0.99%</th>
<th>1.16%</th>
<th>1.33%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW 1 d1</td>
<td>48.7±1.2</td>
<td>48.6±1.2</td>
<td>48.6±1.3</td>
<td>48.4±1.2</td>
<td>48.6±1.0</td>
</tr>
<tr>
<td>BW 3 d2,5,6</td>
<td>9.4±1.0</td>
<td>68.0±6.3</td>
<td>69.9±2.9</td>
<td>70.5±3.5</td>
<td>67.9±8.0</td>
</tr>
<tr>
<td>WG 5,6</td>
<td>-9.8±0.7</td>
<td>18.9±5.9</td>
<td>21.2±3.6</td>
<td>22.1±3.4</td>
<td>19.3±5.7</td>
</tr>
<tr>
<td>PT 7</td>
<td>ND7</td>
<td>23.9±2.0</td>
<td>23.4±1.3</td>
<td>23.9±1.1</td>
<td>22.3±1.7</td>
</tr>
<tr>
<td>SCMA 4</td>
<td>ND7</td>
<td>1.25±0.4</td>
<td>1.10±0.2</td>
<td>1.07±0.1</td>
<td>1.16±0.4</td>
</tr>
</tbody>
</table>

*Values represent mean ± SE. *Diets represent starved=no feed provided, 0.82% lysine, 0.99% lysine, 1.16% lysine, 1.33% lysine.
1Body weight at hatch in grams. 1Body Weight at 3 days of age in grams. 2Means with different superscript are significantly different (P<0.01). 3Weight gain in grams. 4Feed intake in grams. 5Feed Conversion. 6Not Determined.

Table 3: Pectoralis thoracicus (PT) weight, satellite cell mitotic activity (SCMA), and myofiber cross-sectional area (CSA) of broilers fed differing levels of lysine in an ideally balanced feed from 1 to 3 days of age

<table>
<thead>
<tr>
<th>Diets*</th>
<th>Starved</th>
<th>0.82%</th>
<th>0.99%</th>
<th>1.16%</th>
<th>1.33%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT 7</td>
<td>1.2±0.1</td>
<td>3.3±0.7</td>
<td>3.5±0.4</td>
<td>3.5±0.4</td>
<td>3.4±0.6</td>
</tr>
<tr>
<td>SCMA 4</td>
<td>4.2±2.3</td>
<td>11.4±3.5</td>
<td>8.1±2.7</td>
<td>7.0±1.6</td>
<td>7.5±2.6</td>
</tr>
<tr>
<td>CSA 4</td>
<td>20.8±10.6</td>
<td>39.6±14.6</td>
<td>44.3±10.1</td>
<td>41.8±15.9</td>
<td>41.3±17.0</td>
</tr>
</tbody>
</table>

*Values represent mean ± SE. *Pectoralis thoracicus weight grams. 1Satellite cell mitotic activity. The number of BrdU labeled nuclei per 100 total (propidium iodide labeled) nuclei. 2Myofiber cross-sectional area (μm²). 3Diets represent starved=no feed provided, 0.82% lysine, 0.99% lysine, 1.16% lysine, 1.33% lysine.

myonuclear accretion. It is quite interesting that a lysine deficient diet stimulates satellite cell mitotic activity because the expectation is that a higher level of satellite cell mitotic activity results in an increase in myonuclear content, which would ultimately result in an increase in ultimate muscle mass and meat yield at market age. Experimental manipulations that generally increase satellite cell mitotic activity result in increases in muscle size (Darr and Schultz, 1988; Snow, 1990; Dangott et al., 2000). Therefore, it appears that a lysine deficient diet results in a greater number of nuclei available to contribute to myofiber growth, which can ultimately result in a larger muscle at market age. A second potential pathway that nutritional manipulations may influence is the DNA unit or the volume of cytoplasm surrounding each individual nucleus (Allen et al., 1999). Although increases in DNA unit size occur as a consequence of normal muscle growth (Mozdziai et al., 1994; 1997; 2000), DNA unit size does not change following overload induced hypertrophy (McCall et al., 1998). However, it appears that suppressing satellite cell mitotic activity in juvenile muscle causes the muscle to miss a developmental window for myonuclear accretion (Mozdziai et al., 1997; 2000). Therefore, it does not seem possible to cause increases in muscle growth efficiency by inducing changes in the DNA unit size. It appears that increases in DNA unit number through satellite cell fusions are the major determinant of ultimate muscle size. Overall, it appears that myonuclear accretion through satellite cell mitotic activity and fusion is the most important factor during the early post-hatch period determining ultimate muscle size and meat yield in birds (Halevay et al., 2000). However, it is paradoxical that satellite cell mitotic activity was significantly higher (P<0.05) in the birds fed a lysine deficient diet compared to the birds fed higher levels of lysine. Firstly, there has been relatively little study of chick nutritional requirements over the first three days of life because it has been previously generally accepted that the yolk sac provides sufficient nutrients to sustain life over the initial post-hatch period (Turro et al., 1994). While it is true that the yolk sac provides sufficient nutrition to sustain life, it is also quite clear that with-holding feed over the first two to three days of life denies the chick the ability to meet their full genetic potential for skeletal muscle growth and meat yield (Halevay et al., 2000). Growth of the gastrointestinal system requires oral nutrition and gastrointestinal development is retarded in early post-hatch chickens that are not provided feed, even though they have an intact residual yolk sac (Baranyiova and Holman, 1976; Noy et al., 2001; Smirnov et al., 2004; Uni and Ferket, 2004). The major issues of nutritional development of the early post-hatch chick are that the intestinal and metabolic system are not completely mature at hatch. Therefore, it is possible that the early post-hatch bird is not yet able to completely utilize all of the lysine that is present at the recommended lysine levels (National Research Council, 1994), that were likely based upon experiments with birds older than employed in the present study.
Furthermore, it is quite possible that the excess lysine in the feed during the early post-hatch period may reduce skeletal muscle growth potential because excess beneficial amino acids have been shown to reduce chick growth and nutrient utilization (Waldroup et al., 1978). Similarly, the efficient utilization of the lysine at the 0.82% level in the feed may result in a systemic or more likely a localized production of growth factors that stimulate satellite cell mitotic activity, such as IGF-1 and FGF (Dodson et al., 1990). It is likely that the beneficial effects of the lysine-deficient diet are relatively short-term because it is possible that as the intestine matures it can more efficiently absorb lysine. Therefore, the bird may actually exhibit a rapid increase in lysine requirements for an optimal level of satellite cell mitotic activity. It will be important in the future to more precisely correlate satellite cell mitotic activity with dietary lysine to determine the precise levels of lysine that maximize satellite cell mitotic activity throughout production. Once the levels of lysine that maximize satellite cell mitotic activity at each age is designed then it will be possible to maximize meat production.

The overall conclusion from the present study is that a lysine deficient diet during the very early post-hatch period (before 3 days of age) stimulates satellite cell mitotic activity, during the early post-hatch period, which theoretically would lead to an increase in meat yield at market-age. It is important in the future to more clearly define the ages that the lysine deficient diet stimulates satellite cell mitotic activity, and it is also important to better understand nutrient utilization in the early post-hatch chick because it may be significantly different than the adult animal, and the early post-hatch period may also present an important nutritional target to maximize skeletal muscle growth potential.

Acknowledgements
Support was provided, in part, by funds under Project Number NC06590 of North Carolina State University.

References


