Histological Characteristics of Leg Muscles of 56-Day Old Pheasants Hatched from Eggs with Different Eggshell Colour

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Abstract
The aim of this paper was to examine the histological characteristics of leg muscles of pheasants hatched from eggs of a different colour. From muscle samples (M. biceps femoris) of 56-day old pheasants hatched from eggs of different colour (dark brown, light brown, brown/green, blue/green) histological preparations were made. Following parameters were examined: diameter of muscle cells, volume density of connective tissue in muscles and nucleo-cytoplasmatic ratio of muscle cells. Results showed that diameter of muscle cells was smaller in pheasants hatched from blue/green eggs compared to all other examinanted groups. There were no differences in volume density of connective tissue in muscles among groups. Nucleo-cytoplasmatic ratio of muscle cells was higher in pheasants hatched from blue/green eggs compared to all other examinanted groups. From obtained results it can be concluded that pheasants hatched from blue/green eggs had weaker muscle development than pheasants hatched from eggs of other eggshell colour. Cause of this could be related to structural differences of eggshells of various colour. This leads to weaker development of embryos and chicks hatched from blue/green eggs, which reflects on differences in development of leg muscles.

Keywords: eggshell colour, leg muscles, pheasants

1. Introduction
Pheasants are traditionally reared for hunting tourism, but in recent years exists increased need for pheasant rearing because of quality of their meat [1, 2]. One of the characteristics of pheasant development is that chicks hatched from eggs with different eggshell colour. Eggshell can be dark brown, light brown, olive, blue, white, etc. [3, 4]. Embryonic period of development has a great influence on characteristics of postnatal growth [5]. Changes during the incubation can significantly affect the characteristics of postnatal development of tissues and organs [6]. Examinations of processes which occur during the embryonic period of development together with knowledge concerned to characteristics of postnatal growth gives a complete insight of organism development. The aim of this paper was to establish a potential connection between colour of pheasant egg and postnatal development of skeletal musculature of pheasants.

2. Materials and methods
Pheasant eggs were divided in four groups according to eggshell colour: A (dark brown), B (light brown), C (brown/green) and D (blue/green). Each group consisted of 100 eggs.
After hatching, pheasants were raised and on 56th day of life from 5 pheasants of each group sample of leg muscles (M. biceps femoris) were taken. From these samples histological preparations were made according to the procedures described in references [7]. Following parameters were examined: diameter of muscle cells, volume density of connective tissue in muscles and nucleo-cytoplasmatic ratio of muscle cells.

For the analysis of histological preparations classical light microscopy was performed using Leica DMLS equipped with a Leica DC 300 digital camera and the software package IM 1000 (Leica Imaging Systems Ltd, Cambridge, UK). Volume density of connective tissue in muscles and nucleo-cytoplasmatic ratio of muscle cells were determined using the M-42 testing system [8].

Volume density of connective tissue in muscles was calculated using the following formula [6]:

\[ V_v(c_t) = \frac{P(c_t)}{P(m)} \cdot 100(\%) \]

where \( V_v(c_t) \) is the volume density of connective tissue of muscle, \( P(c_t) \) is the number of test points lying over the connective tissue of muscle, and \( P(m) \) is the number of test points lying over the muscle.

The nucleo-cytoplasmatic ratio of muscle cells was calculated using the following formula [6]:

\[ \frac{N/C}{P(n)} = \frac{P(n)}{P(c)} \]

where \( N/C \) is the nucleo-cytoplasmic ratio of muscle cell, \( P(n) \) is the number of test points lying over the cell nucleus, and \( P(c) \) is the number of test points lying over the cell cytoplasm.

Statistical significance was determined using analysis of variance (ANOVA) and post hoc Tukey’s tests for each of the experimental parameters. Statistical tests were carried out using the software package Statistica for Windows, ver. 10.0 (StatSoft, Tulsa, OK, USA).

3. Results and discussion

Results of microscopic examination showed that diameter of muscle cells was smaller (\( p<0.01 \)) in pheasants hatched from blue/green eggs compared to all other examined groups (Figure 1, Table 1). There was no significant difference in volume density of connective tissue in muscles among pheasants hatched from eggs of a different eggshell colour (Figure 1, Table 1). Nucleo-cytoplasmatic ratio of muscle cells was higher (\( p<0.01 \)) in pheasants hatched from blue/green eggs compared to all other examined groups (Table 1).

Smaller diameter of leg muscle cells of pheasants hatched from blue/green eggs can be explained by structural characteristics of blue/green eggshell. Eggshells of blue eggs are thinner and more porous [4], which leads to greater water loss during the incubation and poorer hatchability [9]. This can be related to lower viability of chicks hatched from these eggs, and consequently the weaker development of tissues and organs, such as muscle tissue. This leads to smaller diameter of muscle cells of pheasants hatched from eggs of blue/green eggshell which was obtained in our study.

![Figure 1. M. biceps femoris of pheasants hatched from brown/green (A) and blue/green (B) eggs on day 56 after hatching, H&E staining, scale bar=50μm](image-url)
Table 1. Examined parameters of leg muscle cells (M. biceps femoris) in pheasants hatched from different eggshell colour

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dark brown</th>
<th>Light brown</th>
<th>Brown/green</th>
<th>Blue/green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of muscle cells (μm)</td>
<td>27.58±0.44&lt;sup&gt;A&lt;/sup&gt;</td>
<td>26.80±0.32&lt;sup&gt;A&lt;/sup&gt;</td>
<td>25.93±0.50&lt;sup&gt;A&lt;/sup&gt;</td>
<td>20.06±0.08&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Volume density of connective tissue in muscles (%)</td>
<td>22.37±0.23</td>
<td>22.58±0.78</td>
<td>21.97±0.34</td>
<td>23.25±0.67</td>
</tr>
<tr>
<td>Nucleo-cytoplasmatic ratio of muscle cells</td>
<td>0.22±0.008&lt;sup&gt;H&lt;/sup&gt;</td>
<td>0.24±0.005&lt;sup&gt;H&lt;/sup&gt;</td>
<td>0.24±0.004&lt;sup&gt;H&lt;/sup&gt;</td>
<td>0.28±0.009&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in a row without a common superscript capital letter differ significantly (p<0.01)

Higher nucleo-cytoplasmatic ratio of muscle cells of pheasants hatched from blue/green eggs are related to smaller diameter of these muscle cells compared to muscle cells of pheasants of other groups. With greater diameter of muscle cell, amount of cytoplasm is higher, which leads to smaller ratio between nucleus and cytoplasm inside cell and vice versa. These findings are in line with Ušćebrka et al. [10], who also found similar relation between diameter and nucleo-cytoplasmatic ratio of skeletal muscle cell. Among pheasants hatched from eggs of different eggshell colour there were no differences in volume density of connective tissue in muscles. This can be explained by relation between development of muscle and connective tissue inside muscles. In muscles of pheasants hatched from blue/green eggs, higher number of muscle cells of smaller diameter is formed per unit area compared to muscles of pheasants hatched from all other eggs where smaller number of muscle cells of greater diameter is formed on the same unit area. This results in the approximately the same amount of connective tissue in all examined groups. These observations are similar to findings of Ušćebrka et al. [11], who also detected the same relation between muscle and connective tissue inside muscles. Weaker development of muscle tissue of pheasants hatched from eggs of blue/green eggshell colour determined in our study, can be related to poorer meat quality of these pheasants. Other authors [5, 12] also emphasize importance of muscle development to qualitative and quantitative characteristics of meat.

4. Conclusions

Histological characteristics of leg muscles of 56-day old pheasants differ among pheasants hatched from blue/green eggs compared to pheasants hatched from other eggs. Diameter of leg muscle cells was smaller in blue/green eggs, which pointed out to weaker development of muscle tissue in these pheasants. This can be important in selection of pheasant eggs for incubation.

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References