Characteristics of Skeletal Musculature of Pheasants Hatched from Eggs of Different Eggshell Colour

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Abstract
The aim of this paper was to examine morphodinamics of development of skeletal musculature of pheasants hatched from eggs of different eggshell colour. Four groups of pheasant eggs (dark brown, light brown, brown/green and blue/green) were incubated. Samples of skeletal musculature of leg and breast were taken during the embryonic and neonatal period of development. From taken samples histological preparations were made. In pheasants hatched from blue/green eggs the smaller diameter of leg and breast muscle cells and the higher volume density of connective tissue in leg and breast muscles were recorded. It was concluded that pheasants hatched from blue/green eggs had the weakest development of skeletal musculature, which can be related to structural differences of eggshell of various colour.

Keywords: eggshell colour, pheasant, skeletal musculature

1. Introduction

Examinations of processes which occur during the embryonic and neonatal period of development of birds represent actual topics in recent studies [1, 2]. Having in mind that during the embryonic development of birds intensive physiological and histological processes taking place [3], a plenty of studies confirm that by affecting on those processes standard development patterns can be modified [4]. Consequently, desired changes in development are no more restricted to modification of environmental factors and nutrition during the growth of animals on farms [5].
Pheasants are nowdays mainly breeding for purposes of hunting tourism, especially in countries of rich hunting tradition [6]. Regarding to the needs of market for quality meat, pheasants breeding for these purposes are constantly increasing [7]. Knowledge about embryonic development of pheasants can be used for production of pheasants of high meat quality, health status and vitality of offspring [6].
In pheasant eggs, high variability in eggshell colour of pheasant eggs can be noticed [8, 9]. Pheasant eggshell colour can be dark brown, light brown, olive, blue, white, etc. In previous studies it was pointed out that blue pheasant eggs had low quality characteristics [10], which can be related to slower postnatal development of chickens hatched from these eggs. Some structural abnormalities in blue eggshells were also reported, which can be related to lower quality characteristics of pheasants hatched from those eggs [11].
The aim of this study was to examine the morphodinamics of skeletal muscle development of pheasants chicks hatched from eggs of different eggshell colour and establishing the relation between skeletal muscle development and eggshell colour in pheasants.

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2. Materials and methods

Pheasants eggs were collected and according to eggshell colour divided in four groups: A (dark brown eggshell colour), B (light brown eggshell colour), C (brown/green eggshell colour) and D (blue/green eggshell colour). A total of 400 eggs (100 eggs in each group) of the approximately same weight were selected and set in four 100-egg incubators. In all incubators, eggs were incubated under the standard conditions. For histological examinations, on day 17 of embryonic development (E17) and day 1 after hatching (P1), the samples of leg (*M. biceps femoris*) and breast (*M. pectoralis superficialis*) skeletal muscles were taken. On E17 and P1, samples were taken from 10 pheasants from each group. Muscle samples were fixed in 10% formaldehyde. After fixation, samples were subjected to the standard procedures of dehydration, clearing, embedding in paraffin and cutting into 5 μm thick sections with a microtome. Histological preparations were stained with hematoxylin-eosin (H&E) according to standard procedure [12].

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). Histological preparations were used for determination of diameter and nucleo-cytoplasmatic ratio of skeletal muscle cells as well as volume density of connective tissue in muscles. For determination of nucleo-cytoplasmatic ratio of muscle cells and volume density of connective tissue in muscles, M42 testing system which consists of 21 line segments and 42 points in a testing area was used, according to the procedure described elsewhere [2].

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

$$Vv(ct)=\frac{P(ct)}{P(m)}\cdot 100\ (\%)$$

where Vv(ct) is the volume density of connective tissue of muscle, P(ct) 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:

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

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 two-way 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

Examinations showed that in leg muscles diameter of skeletal muscle cells was smaller ($p<0.05$) in pheasants hatched from blue/green eggs compared to pheasants hatched from brown/green eggs on day 1 after hatching (Figure 1, Table 1). In all groups, diameter of leg muscle cells was greater ($p<0.01$) on day 1 after hatching compared to day 17 of embryonic development (Table 1).

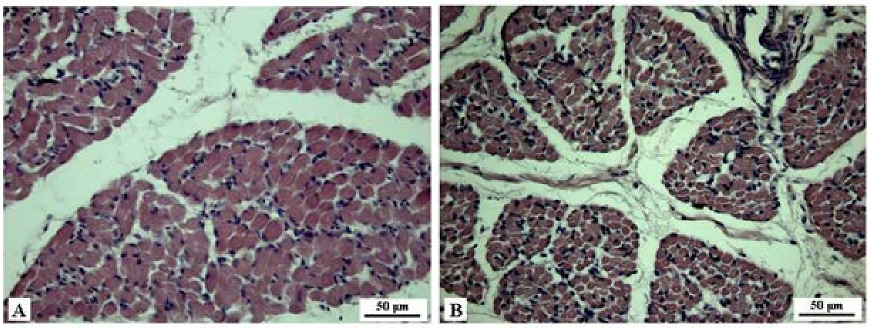

Figure 1. *M. biceps femoris* of pheasants hatched from brown/green (A) and blue/green (B) eggs on day 1 after hatching, H&E staining, scale bar=50μm

Table 1. Diameter (μm) of leg muscle cells (*M. biceps femoris*)

Age (days)	Eggshell colour			
	Dark brown	Light brown	Brown/green	Blue/green
E17	5.98±0.44*	5.80±0.32*	5.53±0.50*	4.67±0.08*
P1	9.15±1.15*	9.09±1.08*	9.56±1.01* [a]	7.81±0.87* [a]
Source	p value			
Eggshell colour (E)	<0.05			
Age (A)	<0.01			
E x A	N.S.			

Means in a row with the same superscript lowercase ([a]) differ significantly (p <0.05)
Means in a column with the same asterisk (*) differ significantly (p <0.01)

On day 17 of embryonic development (Table 2), diameter of breast muscle cells was smaller in pheasants hatched from blue/green eggs compared to pheasants hatched from dark brown eggs (p<0.01), as well as in pheasants hatched from brown/green eggs compared to pheasants hatched from dark brown eggs (p<0.05). On day 1 after hatching (Figure 2, Table 2), diameter of breast muscle cells was smaller in pheasants hatched from blue/green eggs compared to pheasants hatched from light brown eggs (p<0.01). Greater diameter of breast muscle cells was recorded on day 1 of postnatal development compared to day 17 of embryonic development (Table 2) in pheasants hatched from dark brown eggs (p<0.05), as well as in pheasants hatched from all other eggs (p<0.01).

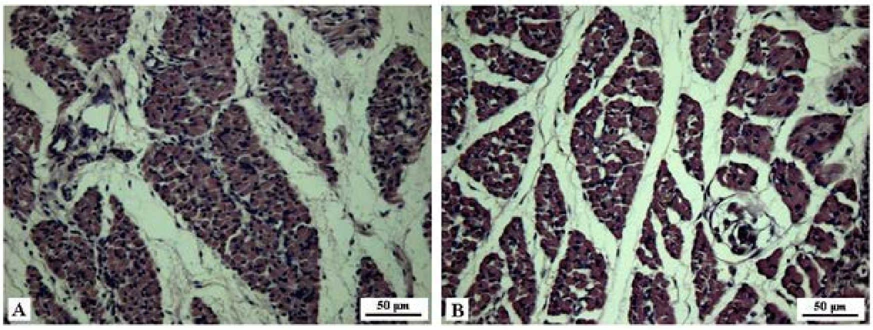

Figure 2. *M. pectoralis superficialis* of pheasants hatched from light brown (A) and blue/green (B) eggs on day 1 after hatching, H&E staining, scale bar=50μm

Table 2. Diameter (μm) of breast muscle cells (*M. pectoralis superficialis*)

Age (days)	Eggshell colour			
	Dark brown	Light brown	Brown/green	Blue/green
E17	4.97±0.35[# aA]	4.55±0.24[*]	4.22±0.19[* a]	3.93±0.09[* A]
P1	5.68±0.32[#]	6.18±0.16[* A]	5.56±0.33[*]	5.24±0.52[* A]
Source	p			
Eggshell colour (E)	<0.01			
Age (A)	<0.01			
E x A	N.S.			

Means in a row with the same superscript lowercase letter ([a]) differ significantly (p<0.05)
Means in a row with the same superscript capital letter ([A]) differ significantly (p <0.01)
Means in a column with the same asterisk ([#]) differ significantly (p<0.05)
Means in a column with the same asterisk (*) differ significantly (p<0.01)

Examinations showed that on day 17 of embryonic development (Table 3) volume density of connective tissue in leg muscles was higher in pheasants hatched from blue/green eggs compared to pheasants hatched from dark brown and light brown eggs (p<0.01), as well as in pheasants hatched from brown/green eggs compared to pheasants hatched from light brown eggs (p<0.01) and dark brown eggs (p<0.05). On day 1 of postnatal development (Figure 1, Figure 2, Table 3), volume density of connective tissue in leg muscles was higher in pheasants hatched from blue/green eggs compared to pheasants hatched from all other eggs (p<0.01). Smaller volume density of connective tissue in leg muscles (Table 3) was recorded on day 1 of postnatal development compared to day 17 of embryonic development in pheasants hatched from light brown and blue/green eggs (p<0.05), as well as in pheasants hatched from dark brown and brown/green eggs (p<0.01).

Table 3. Volume density (%) of connective tissue in leg muscles (*M. biceps femoris*)

Age (days)	Eggshell colour			
	Dark brown	Light brown	Brown/green	Blue/green
E17	$24.00 \pm 2.52^{*\,aA}$	$22.81 \pm 04.45^{\#\,BC}$	$31.14 \pm 2.39^{*\,aB}$	$32.24 \pm 3.39^{\#\,AC}$
P1	$15.96 \pm 3.44^{*\,A}$	$16.15 \pm 2.34^{\#\,B}$	$20.19 \pm 1.83^{*\,C}$	$29.19 \pm 2.04^{\#\,ABC}$
Source	p			
Eggshell colour (E)	<0.01			
Age (A)	<0.01			
E x A	N.S.			

Means in a row with the same superscript lowercase letter (^{a}) differ significantly (p<0.05)
Means in a row with the same superscript capital letter (^{A}) differ significantly (p<0.01)
Means in a column with the same asterisk ($^{\#}$) differ significantly (p<0.05)
Means in a column with the same asterisk (*) differ significantly (p<0.01)

On day 17 of embryonic development (Table 4) volume density of connective tissue in breast muscles was higher in pheasants hatched from blue/green eggs compared to pheasants hatched from dark brown and light brown eggs (p<0.01), as well as in pheasants hatched from brown/green eggs compared to pheasants hatched from light brown eggs (p<0.01) and dark brown eggs (p<0.05). On day 1 of postnatal development (Figure 1, Figure 2, Table 4), volume density of connective tissue in breast muscles was higher in pheasants hatched from blue/green eggs compared to pheasants hatched from all other eggs (p<0.01). Smaller volume density of connective tissue in leg muscles (Table 4) was recorded on day 1 of postnatal development compared to day 17 of embryonic development in pheasants hatched from light brown and blue/green eggs (p<0.05), as well as in pheasants hatched from dark brown and brown/green eggs (p<0.01).

Table 4. Volume density (%) of connective tissue in breast muscles (*M. pectoralis superficialis*)

Age (days)	Eggshell colour			
	Dark brown	Light brown	Brown/green	Blue/green
E17	$25.86 \pm 5.39^{*\,aA}$	$28.99 \pm 2.54^{\#\,BC}$	$33.79 \pm 4.09^{*\,aB}$	$34.24 \pm 3.53^{\#\,AC}$
P1	$21.66 \pm 3.83^{*\,A}$	$23.76 \pm 1.37^{\#\,B}$	$26.09 \pm 3.15^{*\,C}$	$31.43 \pm 1.81^{\#\,ABC}$
Source	p			
Eggshell colour (E)	<0.01			
Age (A)	<0.01			
E x A	N.S.			

Means in a row with the same superscript lowercase letter (^{a}) differ significantly (p<0.05)
Means in a row with the same superscript capital letter (^{A}) differ significantly (p<0.01)
Means in a column with the same asterisk ($^{\#}$) differ significantly (p<0.05)
Means in a column with the same asterisk (*) differ significantly (p<0.01)

Nucleo-cytoplasmatic ratio of leg muscle cells was smaller (p<0.01) on day 1 after hatching compared to the day 17 of embryonic development in all examined groups (Table 5).

Nucleo-cytoplasmatic ratio of breast muscle cells was smaller (p<0.01) on day 1 after hatching compared to the day 17 of embryonic development in all examined groups (Table 6).

Table 5. Nucleo-cytoplasmatic ratio of leg muscle cells (*M. biceps femoris*)

Age (days)	Eggshell colour			
	Dark brown	Light brown	Brown/green	Blue/green
E17	0.67±0.036*	0.64±0.081*	0.72±0.028*	0.66±0.053*
P1	0.18±0.027*	0.20±0.045*	0.19±0.046*	0.30±0.145*
Source	p			
Eggshell colour (E)	<0.01			
Age (A)	<0.01			
E x A	N.S.			

Means in a column with the same asterisk (*) differ significantly ($p<0.01$)

Table 6. Nucleo-cytoplasmatic ratio of breast muscle cells (*M. pectoralis superficialis*)

Age (days)	Eggshell colour			
	Dark brown	Light brown	Brown/green	Blue/green
E17	0.70±0.107*	0.73±0.057*	0.70±0.054*	0.73±0.086*
P1	0.26±0.058*	0.27±0.059*	0.23±0.035*	0.27±0.033*
Source	p			
Eggshell colour (E)	<0.01			
Age (A)	<0.01			
E x A	N.S.			

Means in a column with the same asterisk (*) differ significantly ($p<0.01$)

Analysis showed that diameter of leg muscle cells were smaller in pheasants hatched from blue/green eggs compared to pheasants hatched from brown/green eggs on day 1 after hatching. Also, in breast muscles, on day 17 of embryonic development, diameter of muscle cells was smaller in pheasant embryos from blue/green eggs compared to pheasant embryos from dark brown eggs, while on day 1 after hatching diameter of muscle cells was smaller in pheasants hatched from blue/green eggs compared to pheasants hatched from light brown eggs. These results can be explained by smaller development of skeletal musculature in pheasant embryos from blue/green eggs, which are also reflected on smaller development of these muscles in period after hatching. The cause for this could be related to variability of eggshell structure of pheasant eggs with different eggshell colour. It was detected that eggshell of blue eggs is thinner and more porous [9], which leads to greater water loss during the incubation. Consequently, blue eggs are characterized by poorer hatchability [11]. It can be assumed that chickens hatched from these eggs have lower viability, which can be reflected to weak development of certain tissues and organs. These findings can be related to smaller diameter of skeletal muscle cells of pheasant embryos from blue/green eggs and chickens hatched from these eggs obtained in our study.

Volume density of connective tissue in leg and breast muscles was higher in embryos from blue/green eggs compared to embryos from dark brown and light brown eggs on day 17 of embryonic development, as well as in pheasants hatched from blue/green eggs compared to pheasants hatched from all other eggs on day 1 after hatching. These results can be explained by mutual connection between development of muscle and connective tissue during the muscle development [1]. According to the smaller diameter of skeletal muscle cells in pheasant embryos from blue/green eggs and pheasants hatched from these eggs reported in our study, connective tissue show higher development in these muscles. Consequently, in the same muscle area, volume density of connective tissue in muscles will be higher in muscles in which are skeletal muscle cells weakly developed compared with muscles with greater development of muscle cells. Examination of the connection between muscle and connective tissue in broiler leg and breast muscles showed similar results [3].

Results of this study showed that the weaker development of skeletal musculature was noticed in embryos from blue/green eggs and pheasants hatched from these eggs. This is in agreement with findings that blue eggs have thinner shells with structural defects, while hatchability of these eggs is low [13]. In another study [14], low hatchability of blue eggs was also reported, while these eggs were characterized by the smallest number of blastodermal cells, which are responsible for degree of development of tissues and organs of hatched pheasant chicks. The small amount of these cells could be in line with smaller diameter of skeletal muscle cells of embryos from blue/green eggs and pheasants hatched from these eggs reported in our study.

The development of skeletal musculature in pheasants represents the basic for quantitative and qualitative characteristics of meat in postnatal development, especially at market age [1]. From this point of view, results of this study can be useful for calculation of effectiveness of using blue pheasant eggs for incubation, having in mind that poorer development of skeletal musculature during embryonic period of development and day 1 after hatching could be related with poorer meat quantity and quality in postnatal period of development.

4. Conclusions

Our findings led to the conclusion that characteristics of skeletal musculature of pheasant during the embryonic and neonatal period of development are related to eggshell colour. Examinations showed that these differences are reflected on diameter of leg and breast muscle cells, as well as on volume density of connective tissue in leg and breast muscles. The poorer development of skeletal musculature in embryonic and neonatal period could be reflected on postnatal characteristics of development of skeletal muscles, which could be important for quantitative and qualitative characteristics of pheasant meat.

Acknowledgements

The study was supported by the Provincial Secretariat for Science and Technological Development (Project No. 114-451-678).

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