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## Effect of sex on muscular development of Muscovy ducks

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**Abstract** — Muscovy ducks display marked sexual dimorphism. The aim of our study was to analyse the consequences of dimorphism on muscular growth and, particularly, on the myofibrillar typology of the *Pectoralis major* and *Sartorius* muscles. In the *Pectoralis* muscle, we only found two fibre types: red fast-twitch oxido-glycolytic fibres (about 90 %) and white fast-twitch glycolytic fibres. In the *Sartorius*, the innermost part contained both white (30 %) and red (55 %) fast fibres and red slow-twitch oxidative fibres (15 %). For both muscles, neither sex nor age had a significant effect on the percentage of each fibre type. The cross-sectional areas of fibres increased with age. The difference in muscle weight observed between sexes could be explained by a higher size and/or total fibre number in the male muscles. © Inra/Elsevier, Paris

#### muscle histology / growth / myofibre / duck

**Résumé** — **Effet du sexe sur le développement musculaire du canard de Barbarie.** Le canard de Barbarie présente un dimorphisme sexuel assez prononcé. L'objectif de la présente étude est d'analyser les effets éventuels de ce dimorphisme sur la croissance musculaire et en particulier, sur la typologie myofibrillaire des muscles *Pectoralis major* et *Sartorius*. Le muscle *Pectoralis major* est constitué uniquement de fibres rouges (environ 90 %) de type rapide oxydo-glycolytique et de fibres blanches de type rapide glycolytique. Le muscle *Sartorius* comprend une partie mixte constitué de fibres rouges de type lent oxydatif (environ 15 %), de type rapide oxydo-glycolytique (environ 55 %) et de fibres blanches de type rapide glycolytique (environ 30 %). Ni le sexe, ni l'âge n'ont d'effet significatif sur la proportion de chaque type de fibres dans les deux muscles étudiés. La surface transversale des fibres s'accroît avec l'âge. La différence de poids de muscle observée entre sexes pourrait être due à une taille et/ou un nombre total de fibres musculaires supérieur chez les mâles. © Inra/Elsevier, Paris

#### histologie musculaire / croissance / myofibre / canard

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#### **1. INTRODUCTION**

Sexual dimorphism for body size is observed in almost all domesticated avian species. The degree of divergence between sexes, however, varies considerably between species. In the Pekin duck, the difference is small: 3.5 % [16]. In contrast, in Muscovies, the drake is almost twice the size of the female [16]. Moreover, selection for increased live weight has reinforced this sexual dimorphism [16].

In the Muscovy duck, the difference in body size becomes apparent from 3 weeks of age onwards and increases at a constant rate [11]. Consequently, males and females are always reared separately. After slaughter, males are marketed as cut and boned products, while females are sold, with more difficulty, as whole carcasses. Another important characteristic is the difference in growth rate between sexes. Growth rate is maximum at 30 d of age for the female and 35 d of age for the male [11]. Growth of breast muscle occurs after growth of thigh muscles and is still high for the male after 10 weeks of age [11]. A study of the volumetric growth of muscular fibres from the Sartorius (thigh muscle) showed a divergent evolution between sexes at 7 weeks of age. This growth continued after 10 weeks of age only in the male [19]. This explains the difference of slaughter ages: 10 weeks for the female and 12 weeks for the male.

Rémignon et al. [14] compared two chicken lines derived from a divergent selection based on growth rate. They showed that muscle fibre types (metabolic enzyme profiles, myosin isoform patterns) were not influenced by growth rate and were primarily related to muscle type and function. Divergent selection for high versus low growth rates, however, increases the size and total number of myofibres independently of muscle type [14]. We therefore compared male and female Muscovy ducks from 1 d old to 15 weeks of age to determine the effects of sex and age on the muscular growth and myofibrillar typology of the Pectoralis major and Sartorius muscles.

#### 2. MATERIALS AND METHODS

#### 2.1. Bird management

One hundred male and 100 female Muscovy ducks (Grimaud, Roussay, France), line R51, were reared separately on slats under a red light (5 lux) with a day length of 12 h and a controlled temperature of 20 °C. All ducks received the same diets: a growing diet (12.54 MJ ME·kg<sup>-1</sup> and 190 g CP·kg<sup>-1</sup>) between 0 and 8 weeks of age and a finishing diet (12.74 MJ ME·kg<sup>-1</sup> and 160 g CP·kg<sup>-1</sup>) between 9 and 15 weeks of age. Levery fortnight, from 1 d to 15 weeks, ten males and ten females chosen at random were weighed and slaughtered.

#### 2.2. Histological measurements

Within 10 min post-mortem, two muscles, the Sartorius (SART) and Pectoralis major (PM) were excised and weighed. They were chosen on the basis of their histochemical properties [13, 14]. In each muscle, samples were taken along a line parallel to the fibre axis. All samples were tied, usually at a slightly extended length, to a rubber plate to prevent cold muscle shortening during freezing in isopentane, cooled with liquid nitrogen and stored at -80 °C until histochemical analysis was performed. Serial cross sections, 12  $\mu$ m thick, were obtained at -20 °C. Fibre types were determined on the basis of their ATPase activity after preincubation at pH 4.10 and 10.50 [9] and their succinate dehydrogenase (SDH) activity [1]. Myofibres were classified as types I and II according to the terminology of Barnard et al. [4]. Slow-twitch fibres (type I) were identified as being stable after acid preincubation and labile after alkaline preincubation, while fast-twitch fibres (type II) were labile after acid preincubation and stable after alkaline preincubation. The SDH staining allows the characterisation of fibres with strong oxydative activity (strong blue granulation, type IIa) and weak oxydative activity (pale blue stained fibres, type IIb). The SART muscle can be divided into a fast portion (superficial) composed of type IIa and IIb fibres and a mixed portion (deep) composed of types I, IIa and IIb fibres [20]. We only studied the mixed part. Percentages, mean crosssectional areas (CSA), diameter ratios (DR = smallest diameter/highest diameter), and shape indexes (SI = perimeter<sup>2</sup>/area) of each fibre type were determined using a computerised image analysis system [6]. These parameters were determined on approximately 200 fibres in two random fields for each muscle.

|             | Pectora | ılis major | Sar   | torius  |
|-------------|---------|------------|-------|---------|
| Age (weeks) | Males   | Females    | Males | Females |
| 0           | 0.73    | 0.77       | 0.42  | 0.40    |
| 2           | 0.56    | 0.59       | 0.34  | 0.38    |
| 4           | 0.70    | 0.86       | 0.26  | 0.35    |
| 6           | 1.38 *  | 1.99       | 0.29  | 0.31    |
| 8           | 2.72*   | 3.60       | 0.28  | 0.31    |
| 10          | 4.22*   | 5.39       | 0.23  | 0.26    |
| 12          | 5.72*   | 6.30       | 0.21  | 0.22    |
| 15          | 6.76    | 6.53       | 0.21  | 0.20    |

**Table I.** Sex and age effects on the proportion of *Pectoralis major* and *Sartorius* muscles (%) as compared to body weight of Muscovy ducks (n = 10).

\* For PM, means between males and females differ significantly (P < 0.01).

#### 2.3. Statistical analysis

Data analysis was carried out by variance analysis using the General Linear Model procedure of SAS [15]. The model included the main effects of sex and age. In *table I* and all the figures, the significant (P < 0.01) differences between males and females were indicated with an asterisk (\*). In *tables II* and *III*, the significant (P < 0.05) effect of age, for one parameter, was indicated with different letters following the results.

#### 3. RESULTS

#### 3.1. Growth of ducks and muscles

Mean body weights for males and females were  $44 \pm 4$  g and  $45 \pm 3$  g at 1 d of age, respectively, and reached  $4573 \pm 408$  g and  $2879 \pm 210$  g respectively, at 15 weeks of age. The effect of sexual dimorphism was significant (P < 0.01) on body weight from 4 weeks of age onwards (figure 1), on the weight of SART from 8 weeks of age onwards (figure 2) and on the weight of PM from 10 weeks of age onwards (figure 3). An earlier development of thigh muscles versus breast muscles was observed. In table I, we present the proportion of each muscle as compared to body weight. This proportion increased at a constant rate with age for the PM. For the SART, we observed the opposite situation. Significant differences (P < 0.01) between sexes were found at 6, 8, 10 and 12 weeks of age for the PM.

#### 3.2. Myofibre typology

PM fibre types are presented in table II. The PM was a completely fast-twitch muscle. The mean SI and DR were 2.1 and 0.6, respectively (data not shown). The breast of the Muscovy duck contained about 90 % type IIa fibres and 10 % type IIb fibres (table II). These parameters were not significantly influenced by sex or age. The CSA of the fibres, particularly that of white IIb fibres, increased regularly and significantly with age (P < 0.05). The maximum increase in CSA occurred between 2 and 4 weeks of age ( $\times$  5.6) for type IIa fibres and between 2 and 6 weeks of age  $(\times 18.9)$  for type IIb fibres. The CSA of type IIb fibres was two to three times higher than the CSA of type IIa fibres. At any given age, the CSA of the fibres was generally higher for females than for males, but the difference between sexes was not significant (data not shown).

In the mixed part of the SART, we found slow-twitch type I fibres (15 %) and fasttwitch type IIb fibres (85 %) (*table III*). The percentage of total red fibres (types I and IIa) was high, 70 %, while the percentage of white fibres (type IIb) was low, 30 %.





Figure 2. Variation of *Sartorius* weight with age for male (closed symbols) and female (open symbols) Muscovy ducks (n = 10); \* means a difference between sexes (P < 0.01).

Whatever the fibre type, the mean SI and DR were 2.1 and 0.6, respectively (data not shown). These parameters were not significantly influenced by sex or age. The CSA of the fibres increased regularly and significantly (P < 0.05) until 8 weeks of age, particularly that of white IIb fibres. After 8 weeks of age, we observed a slight increase (type I fibres) or a decrease in the CSA of the fibres.

The evolution of the CSA of type I and IIb fibres was quite similar until 6 weeks of age. After 6 weeks of age, the CSA of type I fibres was higher ( $\times$  1.5). The CSA of type IIb fibres was twice as high as the CSA of type IIa fibres. At hatching, the CSA of the fibres was higher in SART than in PM. At any given age, the CSA of the fibres was generally higher for the males when





compared with the females but the difference between sexes was not significant (data not shown).

#### 4. DISCUSSION

Our study confirmed previous results [11] on the sexual dimorphism of Muscovy ducks and its marked effect on muscular and body growth. We also demonstrated the difference in growth rate between sexes, with females displaying earlier body and muscular development [3]. The PM muscle of Muscovy ducks was entirely fast contracting with mainly red type IIa fibres. In Mallard and Pekin ducks, the proportion of red fibres is lower (85-84 % versus 90 % for Muscovy ducks) and the proportion of white fibres higher (15-16 % versus 10 % for Muscovy ducks) [8, 20]. Conversely, the PM muscle of chickens contains only white type IIb fibres [14]. The shape (DR and SI)

| Pectoralis may | jor(n=20).        |                         | ,                 | 2                       |
|----------------|-------------------|-------------------------|-------------------|-------------------------|
| Age            | Red II            | la fibres               | White             | e IIb fibres            |
| (weeks)        | Percentage        | Area (µm <sup>2</sup> ) | Percentage        | Area (µm <sup>2</sup> ) |
| 0              | 95.7 ± 0.8 a      | 3.1 ± 0.6 f             | $4.3 \pm 0.8$ c   | 9.5 ± 2.3 e             |
| 2              | 87.1 ± 4.5 c      | 9.4 ± 1.5 f             | 12.9 ± 4.5 a      | 22.1 ± 8.9 e            |
| 4              | $90.2 \pm 3.3$ bc | 52.4 ± 17.1 f           | 9.8 ± 3.3 ab      | 99.3 ± 45.0 e           |
| 6              | $89.2 \pm 3.9$ bc | 148.2 ± 46.4 e          | $10.8 \pm 3.9$ ab | 417.3 ± 214.2 d         |
| 8              | 86.6 ± 6.1 c      | 276.0 ± 55.4 d          | 13.4 ± 6.1 a      | 689.6 ± 222.3 c         |
| 10             | 91.2 ± 3.9 b      | 506.9 ± 87.1 c          | 8.8 ± 3.9 ab      | 1 270.8 ± 414.5 b       |
| 12             | $88.8 \pm 6.2$ bc | 648.1 ± 204.5 b         | 11.2 ± 6.2 ab     | 1 418.0 ± 277.8 b       |
| 15             | $90.6 \pm 3.7$ bc | 740.8 ± 117.2 a         | 9.4 ± 3.7 ab      | 1 633.7 ± 533.1 a       |

**Table II.** Age effect on myofibrillar typology (mean  $\pm$  standard deviation) of the Muscovy duck

a, b, ..., f: numbers followed with the same letter in each column are not different (P < 0.05).

| Age (meeks) | R                | ed I fibres                 | Red                       | IIa fibres        | White              | e IIb fibres            |
|-------------|------------------|-----------------------------|---------------------------|-------------------|--------------------|-------------------------|
| (wrrw)      | Percentage       | Area (µm <sup>2</sup> )     | Percentage                | Area $(\mu m^2)$  | Percentage         | Area (µm <sup>2</sup> ) |
| 0           | 15.8 ± 3.2 a     | 105.6 ± 32.2 e              | 57.1 ± 2.5 ab             | 134.3 ± 25.2 e    | 27.1 ± 2.9 bc      | 163.3 ± 48.2 e          |
| 2           | 14.4 ± 3.3 a     | $171.0 \pm 56.1 d$          | 56.3 ± 4.8 ab             | 256.1 ± 55.0 d    | 29.3 ± 4.7 abc     | 354.9 ± 122.3 d         |
| 4           | 16.7 ± 3.7 a     | $452.9 \pm 140.0 \text{ c}$ | 54.4 ± 3.7 ab             | 520.9 ± 84.7 c    | $28.9 \pm 3.2$ abc | $1\ 080.7 \pm 221.8\ c$ |
| 6           | $15.4 \pm 3.4 a$ | 945.7 ± 302.9 b             | 57.8 ± 6.7 a              | 944.6 ± 214.4 b   | 26.7 ± 6.1 c       | 1 886.3 ± 577.3 b       |
| 8           | 14.7 ± 3.3 a     | 1 549.4 ± 408.0 a           | $54.0 \pm 5.1 \text{ ab}$ | 1 128.4 ± 182.0 a | $31.3 \pm 5.2$ abc | 2 275.5 ± 512.1 a       |
| 10          | 16.8 ± 2.9 a     | 1 653.3 ± 446.5 a           | 55.6 ± 6.3 ab             | 1 161.1 ± 260.2 a | $27.6 \pm 5.1$ bc  | 1 989.1 ± 459.2 b       |
| 12          | 14.2 ± 3.5 a     | 1 674.6 ± 607.6 a           | 54.2 ± 5.0 ab             | 1 115.5 ± 305.2 a | $31.5 \pm 5.5$ ab  | 1 844.3 ± 524.7 b       |
| 15          | 14.7 ± 2.4 a     | 1 402.9 ± 417.6 a           | $52.0 \pm 5.2 \text{ b}$  | 912.4 ± 229.7 b   | 33.3 ± 5.2 a       | 1 657.6 ± 404.2 b       |

**Table III.** Age effect on myofibrillar typology (mean  $\pm$  standard deviation) of the Muscovy duck *Sartorius* (n = 20).

a, b, ..., e: numbers followed with the same letter in each column are not different (P < 0.05).

of the cross-section of the fibres was a stable parameter, not influenced by sex, age or muscle type. With age, the CSA of the fibres increased. The higher CSA increase in white fibres was linked to the increase in glycolytic muscular metabolism as demonstrated by Baéza et al. [3] for Muscovy ducks. The activity of lactate dehydrogenase (LDH), an enzyme of glycolytic muscular metabolism, was multiplied by nine between 1 d and 15 weeks of age. In Pekin ducks, the increase in fibre size is similar [8]. In a line of chickens selected for rapid growth, the increase in type IIb fibres occurs earlier [13, 14]. At 11 weeks of age, however, the CSA of chicken fibres is similar to that of Muscovy duck fibres measured at 10 and 12 weeks of age. At any given age, in PM muscle, the CSA of muscular fibres is generally higher in females, who exhibit an earlier growth [3]. Breast weight was, however, higher in males. Thus, the total fibre number and/or their length should be higher in males.

The SART muscle of Muscovy ducks was also predominantly red. In Mallard ducks, the proportion of red fibres is higher (78 % versus 70 %) and the proportion of white fibres, lower (22 % versus 30 %) [20]. The proportion of white IIb fibres was higher in SART muscle when compared with PM muscle. Glycolytic activity is also higher in SART in Muscovy ducks up to 10 weeks of age [3]. In chickens, the proportion of type I fibres is similar; that of type IIa fibres is lower (about 50 %) and that of type IIb is higher (about 35 %) [13, 14]. In duck SART also, the increase in CSA was higher for the type IIb fibres and was linked to an increase in glycolytic muscular metabolism. LDH activity is multiplied by three between 1 d and 15 weeks of age [3]. We therefore confirmed the prevalence of oxidative metabolism at the beginning of postnatal development, progressively replaced by a glycolytic one, as described by Bacou and Vigneron [2] in chicks and Briand et al. [5] in rabbits. After 8 weeks

of age, the CSA of the fibres increased slightly or decreased. This observation was linked to the decrease in SART weight. Swatland [18] showed that the volumetric growth of SART myofibres stops after 8 weeks of age in Pekin and Muscovy ducks. The decrease in CSA of the fibres could be induced by a decrease in locomotor activity. Oishi et al. [12] demonstrated that the immobilisation of rats induces a muscular atrophy. At hatching, the CSA of the fibres was higher in SART than in PM, confirming the earlier development of thigh muscles. In SART, the CSA of the fibres in chickens measured by Rémignon et al. [13, 14] is always smaller than the CSA of the fibres in Muscovy ducks. This difference could explain the reduced tenderness of duck meat compared to chicken meat [17]. A negative correlation between tenderness and muscle fibre size was apparent [7]. At any given age, in Muscovy ducks, the CSA of the SART fibres was generally higher in males and could explain, in part, the difference in muscle weight between males and females. Another hypothesis could be a higher total number and/or length of muscular fibres in males. Henry and Burke [10] compared the weights and the characteristics of three muscles of male and female chicken embryos at 16 and 20 d of incubation. Their data suggest that the muscles of male embryos have more but smaller myofibres than females, which may be responsible for the sex difference in embryo weight and provide the framework for the greater posthatching muscle growth. In Muscovy ducks, the main effects of sexual dimorphism on muscular growth could be a change in the total number and size of myofibres. By counting the total number of myofibres in the Anterior latissimus dorsi, a thin muscle, we could test this hypothesis.

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