ABSTRACT  It was apparent in previous studies at our institution using turkeys that measurements of muscle fibers and extracellular spacing were not adequate to explain what was observed in entire pectoralis major muscle sections. A rating system was developed in which muscle sections were rated from 1 (little extracellular matrix and indistinct muscle fibers) to 5 (large extracellular space and distinct muscle fibers). Maternal inheritance was observed at 16 wk of age but not at 8 or 20 wk of age. The purpose of the current study was to determine the effect of age on maternal inheritance. A line (F) selected long-term for increased 16-wk BW, its randombred control (RBC2), and reciprocal crosses between them were compared from 8 through 18 wk of age. Samples of pectoralis major muscle were obtained in a manner to avoid muscle contraction. After being fixed and cross-sectioned, the muscle samples were stained with hematoxylin and eosin and rated by 4 individuals. No significant difference among genetic groups was observed in scores at 8 wk of age. At 10 wk of age, the F line had lower scores than the other genetic groups. Maternal inheritance was suggested at 12 wk of age. The scores for RBC2 were higher than those for F, whereas the F × RBC2 cross did not differ from the pure RBC2 line score at this age. Although the RBC2 × F scores were higher than the pure F-line scores at 12 wk, they were lower than those of the F × RBC2 crosses. From 14 through 18 wk of age, the scores for the RBC2 line were higher than those for the F line and the maternal inheritance was absolute because the value for the individual crosses did not differ from that of the maternal parent. Based on the results, the type of mating used to produce commercial turkeys would have a major effect on breast muscle morphology from 12 through 18 wk of age.

Key words: turkey, age, breast muscle morphology, inheritance, maternal

INTRODUCTION

The commercial turkey is the result of a cross of a sire line (or sire line cross) and a dam line (or dam line cross). The sire lines are usually selected for increased BW at a given age and muscling, whereas the dam lines are selected for growth and reproduction traits. Growth, feed conversion, and muscling have been greatly improved by commercial turkey breeders (Nestor et al., 1969; Havenstein et al., 2004a,b). However, the turkey processing industry is experiencing a meat quality problem that is similar to the pale, soft, and exudative condition in swine (Sosnicki and Wilson, 1991). Velleman et al. (2003a) studied development of the turkey pectoralis major muscle in a randombred control line (RBC2), a subline (F) of the RBC2 line selected long-term only for increased 16-wk BW, and a commercial sire line (B) at 25 d of incubation and at 1, 4, 8, 16, and 20 wk posthatch. The RBC2 line maintained well-organized muscle fibers and muscle fiber bundles with large capillary networks throughout the duration of the study. In contrast, the growth-selected F line began to show muscle fiber degeneration at 8 wk posthatch, and limited capillary beds were observed as development proceeded. The B line had intermediate muscle morphology between the RBC2 and F lines, but by 20 wk posthatch, significant muscle fiber degeneration was present with limited capillary supply. The degenerative muscle fiber changes were predominant in the growth-selected F line, suggesting that single-trait selection for increased BW may be associated with posthatch muscle damage.

The inheritance of various measurements of muscle development in the turkey during the embryonic pe-
MATERIALS AND METHODS

Genetic Stocks and Matings

The RBC2 line, the base population of the F line, was started in 1966 from reciprocal crosses of 2 commercial strains that were representative of commercial turkeys at the time (Nestor et al., 1969) and has been maintained by random mating without intentional selection using a paired mating system (Nestor, 1977a) with 36 parental pairs. Little genetic change over generations in the RBC2 line has been expected or observed (Nestor, 1977b; Noble et al., 1995). Details of the maintenance of the F line and response to selection have been previously reported (Nestor, 1977c, 1984; Nestor et al., 1996, 2000, 2008). The F line had been selected for increased BW of the F line was approximately double or more than double that of the RBC2 line at 8, 16, and 20 wk of age and at sexual maturity (females only) (Nestor et al., 2008).

Offspring from the pure F and RBC2 lines were obtained from the matings used to reproduce the lines. For the F line, 36 sires were artificially mated to 72 dams with each sire assigned to 2 dams. The RBC2 line was reproduced by 36 parental pairs. To produce the reciprocal crosses, 52 RBC2 and 45 F-line dams were used. The sires used to produce the pure lines were also used to produce the reciprocal crosses. Each sire was individually mated to 1 or 2 dams. When referring to the reciprocal crosses, the line of the sire is listed first. Samples of the pectoralis major muscle were obtained at 8, 10, 12, 14, 16, and 18 wk of age from 10 males and 10 females of each genetic group. Offspring from the study were produced in a single 3-wk hatch.

Management of Birds

The birds were grown, sexes separate, in confinement in separate houses with the genetic groups intermingled. All birds were provided a declining protein, 5-ratio system (Naber and Touchburn, 1970) for consumption ad libitum based on the schedule for males. The different diets met or exceeded NRC standards. Continuous lighting was provided from hatching to 8 wk of age, when the photoperiod was reduced to 12 h/d and remained at this level until the end of the experiment.

Muscle Measurements

Birds were killed at all ages by cervical dislocation with restraint to prevent flapping of the wings. After immediate removal of the skin from the breast region, a sample of the pectoralis major muscle was obtained by carefully dissecting approximately 0.5 cm of the muscle fibers following the orientation of the muscle fibers for a length of about 3 cm. The ends of the muscle fibers were tied to wooden sticks by surgical thread before removal to prevent muscle contraction.

The muscle samples were fixed and stained as described previously (Velleman and Nestor, 2004). The stained muscle sections were analyzed for muscle morphology with an Olympus XI 70 microscope equipped with an Olympus Magna Fire digital camera linked (Olympus, Melville, NY) to a computer with Image Pro software (Media Cybernetics, Silver Spring, MD). Each slide from each bird contained a minimum of 4 sections and 5 fields of each section were evaluated. Representative sections were rated 1 to 5 based on breast muscle morphology as previously reported by Velleman et al. (2003c). A rating of 1 indicated little extracellular space and indistinct muscle fibers, whereas a rating of 5 indicated a large extracellular space and distinct muscle fibers. Ratings of 2 to 4 were intermediate to these extremes.

Statistical Analysis

The statistical analysis was based on the mean morphology score of 4 individual raters. The data were analyzed within age with genetic group (pure lines and reciprocal crosses), sex, and the interaction of genetic group and sex as sources of variation. Means were separated by repeated t-tests.
RESULTS

The breast morphology scores across all genetic groups did not differ between sexes at any age (data not shown); therefore, means for sexes combined were compared. At 8 wk of age, there was no significant difference among the genetic groups (Table 1). At 10 wk of age, the pure F line had significantly lower breast morphology scores than the pure RBC2 line and the reciprocal crosses did not differ. At 12 wk of age, maternal inheritance started to appear because scores for the pure RBC2 line (3.63) did not differ from the F × RBC2 cross (3.44), but both were larger than those for the pure F line and RBC2 × F cross. However, the pure F line had a significantly lower score (2.22) than the RBC2 × F cross (2.68). The comparisons at 12 wk of age were complicated by an interaction (\(P < 0.05\)) of genetic group and sex. From 14 through 18 wk of age, maternal inheritance was absolute within age in the sense that the pure lines differed in morphology scores and the morphology scores of reciprocal crosses did not differ from that of the maternal parent.

The interaction means of genetic group and sex at 12 wk of age are presented in Table 2. The significant interaction resulted from there being no sex difference in morphology scores for the pure F line, the females having higher scores than males for the pure RBC2 line and RBC2 × F cross and the males having higher scores than the females in the F × RBC2 cross.

Figures 1, 2, and 3 contain representative pectoralis major muscle histological samples of turkeys from the F × F, RBC2 × RBC2, RBC2 × F, and F × RBC2 matings at 10, 12, and 14 wk of age, respectively. At 10 wk of age (Figure 1), only the F × F pectoralis major muscle showed muscle fiber fragmentation and reduced connective tissue spacing. At 12 wk of age (Figure 2), the RBC2 × F reciprocal cross resembled the F × F muscle morphological structure. In contrast, in the F × RBC2 reciprocal cross muscle structure was similar to the RBC2 × RBC2 muscle. The maternal inheritance of the pectoralis major muscle morphological structure continued throughout the duration of the study. Figure 3 contains representative histological samples of the pectoralis major muscle from each of the crosses, illustrating the maternal influence on muscle fiber organization.

DISCUSSION

Breast muscle morphology in turkeys is influenced by BW. In the present study, the F line, selected only for increased 16-wk BW, had a lower breast muscle morphology score than the RBC2 line, the base population of the F line, beginning at 10 wk of age. The breast

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>F × F</th>
<th>RBC2 × RBC2</th>
<th>F × RBC2</th>
<th>RBC2 × F</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>3.60</td>
<td>3.64</td>
<td>3.68</td>
<td>3.87</td>
</tr>
<tr>
<td>10</td>
<td>2.29b</td>
<td>3.44a</td>
<td>3.84a</td>
<td>3.54a</td>
</tr>
<tr>
<td>12</td>
<td>2.22c</td>
<td>3.63a</td>
<td>3.44a</td>
<td>2.68b</td>
</tr>
<tr>
<td>14</td>
<td>2.50b</td>
<td>3.80a</td>
<td>3.44a</td>
<td>2.92b</td>
</tr>
<tr>
<td>16</td>
<td>2.84b</td>
<td>3.85a</td>
<td>3.58a</td>
<td>2.78b</td>
</tr>
<tr>
<td>18</td>
<td>2.34b</td>
<td>3.78a</td>
<td>3.38a</td>
<td>2.58b</td>
</tr>
</tbody>
</table>

a–cMeans within ages with no common superscript differ (\(P < 0.05\)).

Table 2. Interaction of genetic group and sex on breast muscle morphology scores\(^1\) at 12 wk of age

<table>
<thead>
<tr>
<th>Sex</th>
<th>F × F</th>
<th>RBC2 × RBC2</th>
<th>F × RBC2</th>
<th>RBC2 × F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2.12d</td>
<td>3.34b</td>
<td>3.70a</td>
<td>2.36d</td>
</tr>
<tr>
<td>Female</td>
<td>2.31d</td>
<td>3.93c</td>
<td>3.18c</td>
<td>3.02c</td>
</tr>
</tbody>
</table>

a–dMeans with no common superscript differ (\(P \leq 0.05\)).

1Muscle sections were subjectively rated by 4 individuals and statistical analysis was based on the means. The ratings ranged from 1 (little extracellular matrix and indistinct muscle fibers) to 5 (large extracellular space and distinct muscle fibers). Ratings of 2 to 4 were intermediate to these extremes.

2Sires lines are listed first.

3There was a significant (\(P < 0.05\)) interaction between genetic group and sex.
muscle scores of the F line remained relatively constant from 10 through 18 wk of age, whereas those of the RBC2 line increased from 10 through 14 wk of age and remained relatively constant thereafter.

Selection for increased total BW in the F line increased the total weight of the pectoralis major muscles at 4, 8, 12, and 16 wk of age (Lilburn and Nestor, 1991) and the breast muscle percentage of total BW at 16 wk of age was greater (Nestor et al., 1988) relative to the RBC2 line. In the study of Lilburn and Nestor (1991), a commercial sire line (C) was included in the study. The C line was smaller than the F line at 4 through 12 wk of age but was larger than the F line at 16 wk of age. The pectoralis major muscles of the C line were heavier than those of the F line from 8 through 16 wk of age, suggesting that selection emphasis in the C line was placed on breast width in addition to BW. Velleman et al. (2003c) reported that another sire line (B) had larger extracellular spacing than the F line based on breast muscle morphology scores. The selection criteria of the B line were unknown, but selection was likely placed on BW and breast width.

Because the muscle fibers are more dense than the material in the extracellular spaces, it is interesting to speculate that selection for increased BW at a specific age would result in an increase in muscle fibers and a reduction in extracellular spacing, as was seen in the F line relative to the RBC2 line in several studies at 16 wk of age (Velleman et al., 2003b,c; Velleman and Nestor, 2004, 2006). On the other hand, if the production of muscle fibers required more biological energy than production of the extracellular spacing, and selection in commercial sire lines was for both increased BW and breast width, it would be more biologically efficient for the commercial sire lines to increase extracellular spacing more rapidly than muscle fiber size to increase breast width. This would result in higher breast muscle morphology scores than were observed in the F line as reported by Velleman et al. (2003c).

Long-term selection for increased egg production in turkeys resulted in a decline in BW and of extracellular spacing relative to a randombred control that served as the base population for the egg line (Velleman and Nestor, 2006). Velleman and Nestor (2006) speculated that similar changes in breast muscle morphology by increases in BW in the F line and decreases in BW of the egg line may be due to selection influencing genetic homeostasis, which is defined as the property of populations to equilibrate their genetic composition to resist sudden changes (Lerner, 1954). One contributing mechanism to genetic homeostasis is the superiority with respect to fitness of the heterozygote over

Figure 1. Representative pectoralis major muscle sections at 10 wk of age for A) F sire × F dam, B) RBC2 sire × RBC2 dam, C) RBC2 sire × F dam, and D) F sire × RBC2 dam. RBC2 = randombred control line and F = subline of RBC2 selected long term for increased 16-wk BW. Magnification bar = 50 μm.
the homozygote (Lerner, 1954). It has been well established that long-term selection decreases the frequency of heterozygotes (Falconer, 1982).

There was an interaction between genetic group and sex at 12 wk of age in the current study. In previous studies, the sexes did not differ, nor were there any interactions between sex and genetic group, for breast muscle morphology scores at 16 wk of age (Velleman et al., 2003c; Velleman and Nestor, 2004). The interaction of genetic group and sex in the present study may have been due to chance sampling or to a different rate of development in the different genetic groups. There was no significant interaction between these variables at 8, 10, 14, 16, and 18 wk of age.

Maternal inheritance of breast muscle morphology scores in turkeys has been observed previously at 16 wk of age in crosses of the F and B lines, the F and RBC2 lines, and a line selected for increased egg production and its randombred control (Velleman et al., 2003c; Velleman and Nestor, 2004, 2006). In all but one comparison, the maternal inheritance was absolute in that the values for the reciprocal cross did not differ significantly from that of the maternal parent. Similar results were observed in the current study from 14 through 18 wk of age. At 12 wk of age, scores for the pure RBC2 line did not differ from those of the F × RBC2 line cross, but the value for the RBC2 × F cross was significantly larger than the value for the pure F line. Maternal inheritance of breast muscle morphology has not been observed in previous studies at 8 or 20 wk of age (Velleman and Nestor, unpublished data). The lack of maternal inheritance at 20 wk of age may be due to the inability to accurately evaluate muscle sections because of extensive fiber fragmentation and degeneration at this age in growth-selected birds (Velleman et al., 2003a).

Differences between reciprocal crosses can be due to sex-linked or maternal inheritance. Because the results were similar for male and female offspring in studies of Velleman et al. (2003a), Velleman and Nestor (2004, 2006), and in the present study, sex-linked inheritance was not involved. The results of the current study and those of Velleman et al. (2003a) and Velleman and Nestor (2004, 2006) indicate strong maternal inheritance of breast muscle morphology. Maternal inheritance has previously been reported in poultry. In special backcrosses, Allen (1962) reported that the plasmon (stable properties of the cytoplasm that may be transmitted to the offspring) affected pullet mortality from zygote to 360 d after hatching, visceral lymphomatosis mortality occurring between 150 and 360 d of age, hen-day egg production, egg weight, days to first egg, blood spot scores, meat spot scores, albumen firmness, and shell color, but not egg shape. Maternal inheritance can

Figure 2. Representative pectoralis major muscle sections at 12 wk of age for A) F sire × F dam, B) RBC2 sire × RBC2 dam, C) RBC2 sire × F dam, and D) F sire × RBC2 dam. RBC2 = randombred control line and F = subline of RBC2 selected long-term for increased 16-wk BW. Magnification bar = 50 μm.
result from 1) mitochondrial inheritance, 2) epigenetics or genetic imprinting, and 3) egg factors.

A review of mitochondrial inheritance was published by Birky (2001). Mitochondrial inheritance has been shown to occur in dairy cattle (Ashley et al., 1989; Koebrcher et al., 1991) and mice (Jenuth et al., 1996, 1997; Chinnery et al., 2000). The mechanisms involved in the regulation of mitochondrial inheritance in animals are still obscure (Moraes, 2001).

Genetic imprinting has been shown to occur in angiosperms, mammals, and some protozoa (Pennisi, 2001), but not in birds (Moore and Haig, 1991). More than 40 imprinted genes have been found, about half are expressed when they come from the sire and half when they come from the dam (Pennisi, 2001). If genetic imprinting was of importance in the inheritance of breast muscle morphology, some paternal inheritance should have been observed but was not (Velleman et al., 2003a; Velleman and Nestor, 2004, 2006; present study).

It is well known that egg traits of turkeys influence performance posthatch. As an example, egg weight has been shown to influence BW up to 24 wk of age in some studies (Scott and Phillips, 1936; Bray, 1965). Quite likely, differences in egg traits such as total egg weight were not responsible for the maternal inheritance that occurred using an egg line with small eggs (Velleman and Nestor, 2006) and growth lines with large eggs (Velleman et al., 2003a; Velleman and Nestor, 2004).

No matter what the cause of the maternal inheritance, the type of mating used in the production of commercial crosses would have a major effect on performance. Lines with desirable characteristics in breast muscle morphology should be used as the female parent for commercial production. Based on the results of the current study, phenotypic expression of maternal inheritance of breast muscle morphology is expected to occur from 12 through 18 wk of age.

REFERENCES