# Comparison of Chicken Genotypes: Myofiber Number in Pectoralis Muscle and Myostatin Ontogeny

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**ABSTRACT** This study was performed to evaluate breast muscle development in chicken genotypes divergently selected for muscularity. In the first experiment, 2 commercial broiler lines (a high breast yield, HBY, and a normal breast yield broiler strain-cross, NBY) and a Leghorn line were grown up to 35 d to evaluate BW, breast weight, and breast yield. At 7 and 21 d of age, pectoralis muscle was used to estimate myofiber density (MFD, number of myofibers per $mm^2$) and total apparent myofiber number (MFN). In the second experiment, the ontogeny of myostatin was determined from broiler- and Leghorn-type chick embryos, at embryonic days 1 to 20 (E1 to E20), using reverse transcription (RT)-PCR. As expected, the Leghorn line had lower BW, breast weight, and breast yield than broiler lines. The HBY line showed higher breast yield at all ages evaluated, but lower BW at 21 and 35 d than the NBY line. The Leghorn line had 45% higher MFD than broilers, which indicates an increased cross-sectional area of the myofibers in broiler lines. No MFD difference was observed between the broiler strains ($P > 0.05$). The myofiber number of broilers was more than twice that of Leghorns and HBY had 10% higher MFN than the NBY line. Myofiber number was correlated to BW (r = 0.58), breast weight (r = 0.58), and breast yield (r = 0.69). Conversely, MFD showed negative correlation with BW, breast weight, and breast yield (r = –0.85, –0.83, and –0.88, respectively). No effect of genotype or interaction between genotype and embryonic age was observed for myostatin expression. This study showed that broilers have higher MFN in the breast muscles than Leghorn-type chickens, and that high breast yield of broiler strains may be due to increased MFN. Higher muscularity of broilers, as compared with Leghorns, was not attributed to lower expression of myostatin during embryonic development.

(*Key words*: broiler, myostatin, breast yield, myofiber number)

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## INTRODUCTION

The economic importance of poultry white meat has driven extensive genetic selection efforts to increase breast yield of commercial broiler chicken genotypes. In fact, genetic selection programs, with the help from improvements in production systems, have been extraordinarily successful in increasing the meat yield of broiler chickens. Although such improvements in muscularity are not clearly understood physiologically, they most likely involve the quantity or size of myofibers.

Considering the fact that myofibers are the major component of the muscle tissue, many studies have attempted to relate the myofiber characteristics and number to muscularity in different domestic animal species. Double-muscled cattle, which are highly muscular, have been shown to possess about twice as many myofibers as normal cattle (Swattland and Kiefer, 1974). Similarly, in pigs, a positive relationship of myofiber number (MFN) to fast growth rate has been demonstrated (Dwyer and Stickland, 1991; Dwyer et al., 1993, 1994). In poultry, when growth-selected chicken genotypes are compared with laying or Bantam chickens, the former have shown much higher muscularity and pronounced hypertrophy (Aberle et al., 1979; Burke and Henry, 1997). Burke and Henry (1997) also observed that, at hatch, commercial broilers had twice as many myofibers as Bantam chickens in the semimembranosus muscle. Remignon et al. (1994, 1995) reported that fast-growing chicken lines had 15 to 20% more myofibers in the anterior latissimus dorsi muscle (a red type muscle) than slow-growing chicken lines. Such differences in the MFN have not been clearly demon-



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**Abbreviation Key:** HBY = high breast yield broiler strain-cross; MFD = myofiber density; MFN = total apparent myofiber number of a muscle; NBY = normal breast yield broiler strain-cross; RT = reverse transcription.

strated for the breast muscles because of methodological difficulty in estimating the myofiber number in pennate-oriented myofibers. However, if MFN in the pectoralis muscle could be estimated and correlated to ultimate breast yield, it could potentially be used to explain yield differences among the commercially available broiler strains.

Muscle development in avian species occurs in 2 distinctive periods. First, during the embryonic phase, the MFN is established when a large number of precursor cells are committed to the expression of muscle-specific genes (Christ and Brand-Saberi, 2002). Later, during the posthatch period, the hypertrophy of the muscle takes place, mainly by accretion of protein and nuclei originating from the proliferation and fusion of satellite cells (Moss, 1968). Embryonic muscle growth is the result of a balance between proliferation and differentiation of myoblasts into myotubes, such that an increased myoblast proliferation rate is expected to increase the MFN, resulting in stimulation of muscle growth and enlargement of muscle size (Coutinho et al., 1993; Thomas et al., 2000; Christ and Brand-Saberi, 2002). Higher myoblast proliferation rate might be related to the timing in myogenesis, as delay in the appearance of the somites and the expression of myogenic regulatory factors and myosin heavy chain was observed for growth-selected quail when compared with control lines (Coutinho et al., 1993). Similarly, fetal double-muscled cattle showed a delay in myoblast differentiation compared with normal-muscled cattle (Picard et al., 1995), indicating that a prolonged proliferation period might result in more myoblasts, higher number of myotubes, and more fully differentiated myofibers.

The myogenic program is regulated by the myogenic transcription factors that are regulated by hormones and growth factors such as insulin-like growth factors (IGF), fibroblast growth factors (FGF), and transforming growth factors (TGF). Myostatin, a member of the TGF-$\beta$ family of growth factors, has been shown to be a negative regulator of muscle mass through involvement in the myogenic regulatory gene pathway (McPherron et al., 1997; Lee and McPherron, 2001). Transgenic mice lacking a functional myostatin gene exhibit a 2- to 3-fold greater muscle mass than wild-type mice, due to hyperplasia and hypertrophy of muscle fibers (McPherron et al., 1997), whereas double-muscled cattle have mutations in the myostatin coding sequence (Kambadur et al., 1997; McPherron and Lee, 1997; Grobet et al., 1998). The sequence and function of myostatin appears to be highly conserved among vertebrate species, such that its role in chicken myogenesis is expected to be similar to that observed in mammals. For example, it has been reported that in chicken embryos, myostatin down-regulates Pax-3, a gene associated with proliferation of myogenic cells, and prevents the expression of Myo-D, a gene associated with the activation of the myogenic program (Amthor et al., 2002).

A temporospatial profile of myostatin expression in chicken was reported by Amthor et al. (2002), showing the expression of the gene in the central part of the dermomyotome and in the myotome a considerable time after the formation of the sclerotome and myotome. Myostatin expression in chickens was also analyzed temporally by reverse transcription (RT)-PCR (Kocamis et al., 1999) and a comparison between broilers and Leghorns at d 1 of embryonic age and d 10 posthatch was also reported (Mott and Ivarie, 2002). Because the growth rate and muscularity of broiler- and Leghorn-type chickens is different, these strains provide a suitable model for studying myogenic gene expression during the embryonic period. The comparison of the myostatin ontogeny during the whole embryonic period between chicken genotypes with different muscularity could show potential windows of development where higher expressions would be expected in Leghorn-type chickens. During such periods, potential external inhibition or down-regulation of myostatin through introduction of pharmacological substances would be of special research interest.

The objectives of this study were: 1) to compare 2 broiler strain-crosses and a Leghorn-type chicken line based on the apparent number of myofibers in the pectoralis muscle, and 2) to determine if the difference in muscularity between broiler- and Leghorn-type chickens may be related to the ontogeny of myostatin mRNA expression during the embryonic development.

## MATERIALS AND METHODS

### *Myofiber Number Study*

For this experiment, day-old sexed chicks from 1 Leghorn- and 2 broiler-type commercial genotypes were obtained from commercial sources. The broiler genotypes were known to have different breast yields and are here referred to as high breast yield (HBY) and normal breast yield (NBY). The chickens were raised in Petersime batteries up to 35 d of age on a common commercial broiler feed. Feed and water were provided ad libitum.

Weekly, a sample of 24 birds per genotype was used to determine BW and pectoralis muscle weights. At 7 and 21 d of age, chicks were euthanized with $CO_2$, and the pectoralis muscle on one side of the breast was excised and weighed. The other side of the breast was used for myofiber density (MFD) and MFN determinations, based on the method by Burke and Henry (1997) with some modifications. Pectoralis muscle, still attached to the bone, was cross-sectioned perpendicular to the direction of the myofibers (Figure 1) and the cross-sectional area was traced on transparent paper. A tissue block of the pectoralis superficialis muscle was removed, mounted on a cork coated with gum tragacanth, frozen in isopentane, cooled in a liquid nitrogen bath, and stored at –80°C. Tissue sections of 10 $\mu$m were prepared with a cryostat at –20°C and stained with hematoxylin and eosin. Myofibers were enumerated on a microscope (Axivert ZEISS-200) equipped with a digital analyzer system. Five random fields were counted on each slide and the average generated. The total apparent number of myofibers (MFN) was estimated by multiplying the average number of myofibers on the microscope field area by the total cross-sec-

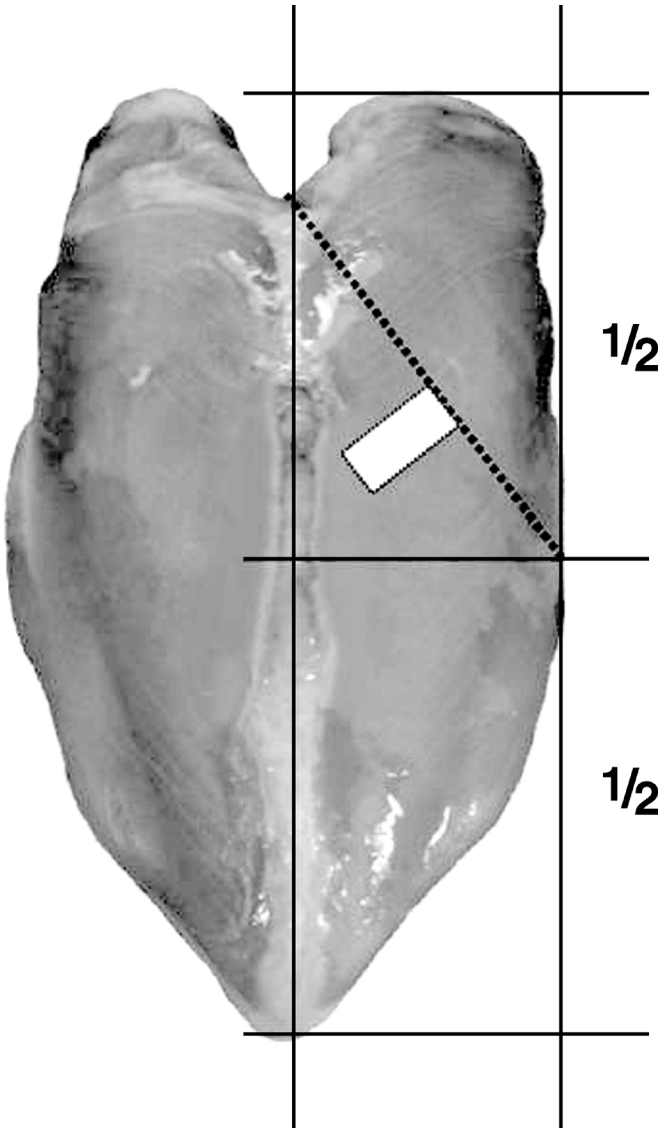


**FIGURE 1.** Schematic view of pectoralis muscle sampling. Pectoralis muscle, still attached to the bone, was cross-sectioned perpendicular to the orientation of the myofibers (----), and the cross sectional area was traced on a transparent paper. A tissue block of the pectoralis superficialis muscle was removed for estimation of total apparent myofiber number and myofiber density.

tional area of the muscle, which was obtained using an Ott planimeter.

### *Myostatin Ontogeny Study*

In this experiment, the ontogeny of myostatin was determined during the embryonic development in both broiler- and Leghorn-type chickens. Fertile eggs from commercial broiler- and Leghorn-breeders were obtained from commercial sources and incubated at 37.5°C and 60% RH. Nine embryos per line were sampled daily and stored in Trizol reagent[2] at –80°C in pools of 3 embryos. The embryo samples varied by age: from embryonic ages d 1 to 5 (E1 to E5), the whole embryo was sampled; from E6 to E8, decapitated embryos were used; and from E9 to E20, only the pectoralis muscles were used.

RNA was isolated using Trizol reagent[2] according to the manufacturer's recommendations. The concentration of RNA was estimated based on the absorbance at 260 nm, using a spectrophotometer.[3] Quality of RNA was assured by checking the integrity through agarose gel electrophoresis and by confirming that the absorbance ratio $A_{260nm}$:$A_{280nm}$ was between 1.8 and 2.0 (Sambrook and Russell, 2001).

Myostatin expression was estimated through a semi-quantitative RT-PCR procedure using a Qiagen one-step RT-PCR kit.[4] A volume of 25 $\mu$L was used in the PCR tubes, with a concentration of 400 $\mu M$ of each dNTP, 2.5 m$M$ $MgCl_2$, 0.8 m$M$ of each primer, 1 $\mu$L of enzyme mix, and 0.01 $\mu$g of total RNA template, which was established in a preliminary trial. Primers used for myostatin (fragment positioned at 324 to 574, with 250 bp) were GATGACTATCATGCCACAACCGAGA (forward) and TGCCTGGGTTCATGTCAAGTTTCA (antisense). To express myostatin expression as relative densitometry, a fragment of 18S rRNA was also amplified using primers: CTAGTTAGCATGCCGAGAGT (forward) and CTAGTTAGCATGCCGAGAGT (antisense). Reverse transcription-PCR was performed on a PTC-100TM thermal cycler[5] with 30 min at 50°C for reverse transcription, followed by 15 min at 95°C for activation of the PCR enzymes. Thirty-two cycles of the following program were performed: denaturation (1 min at 94°C), annealing (30 s at 52°C), and extension (1 min at 72°C), with a final 10 min at 72°C for extension. For the first 7 cycles, a touch-down annealing temperature was used, starting with 65°C and reducing by 2°C per cycle until the annealing temperature of 52°C was reached. A volume of 15 $\mu$L of the PCR product was electrophoresed (1.5% agarose gel) producing a single band. The gel was scanned on a Gel-Doc 1000 scanner[6] and the densitometric analysis was performed with Quantity One software[5] with 3 variables: volume (intensity × area), area ($mm^2$), and density (intensity).

A preliminary trial was performed to evaluate the efficacy of the RT-PCR program and the densitometric analysis in response to increased RNA template, and to establish the RNA template quantity to be used. The preliminary trial was designed to express the gene relative to 18S rRNA. A constant quantity of total RNA (chicken muscle RNA + pig muscle RNA) was kept in the tubes, but quantities of chicken RNA increased whereas pig RNA decreased. Myostatin primers were specific for chicken RNA, whereas 18S rRNA primers detected both chicken and pig RNA, such that increased relative densitometric values of myostatin were expected with higher chicken RNA template.

Statistical analysis of the data was performed using the GLM procedure of SAS (1989) considering genotype and

[2]Life Technologies-Invitrogen, Carlsbad, CA.
[3]Thermo Spectronic – Genesys8, Rochester, NY.
[4]Qiagen Inc., Valencia, CA.
[5]MJ Research, Inc., Waltham, MA.
[6]Bio-Rad Laboratories, Hercules, CA.

**TABLE 1. BW and characteristics of pectoralis muscles of broiler- and Leghorn-type chickens at 7 d of age[1]**

| | BW (g) | Breast weight[2] (g) | Breast yield[3] (%) | Pectoralis area ($mm^2$) | MFD[4] | MFN[5] |
|---|---|---|---|---|---|---|
| Females | 165.7[b] | 14.5[b] | 8.24 | 383.7[b] | 448.4 | 152,288[b] |
| Males | 175.6[a] | 15.5[a] | 8.39 | 411.8[a] | 427.0 | 168,940[a] |
| SEM | 2.6 | 0.3 | 0.13 | 9.2 | 13.0 | 5,437 |
| Line[6] | | | | | | |
| HBY | 203.7[a] | 20.6[a] | 10.10[a] | 547.7[a] | 379.0[b] | 207,610[a] |
| NBY | 210.4[a] | 18.3[b] | 8.73[b] | 489.9[b] | 389.0[b] | 188,393[b] |
| Leghorn | 97.9[b] | 6.0[c] | 6.13[c] | 155.6[c] | 549.1[a] | 85,837[c] |
| SEM | 3.1 | 0.4 | 0.16 | 12.7 | 16.0 | 6,657 |
| CV (%) | 8.22 | 12.16 | 8.4 | 12.6 | 15.6 | 17.5 |
| Line | *** | *** | *** | *** | *** | *** |
| Sex | ** | * | NS | * | NS | * |
| Sex × line | NS | NS | NS | * | ** | ** |

[a-c]Means within columns with no common letter differ significantly ($P < 0.05$).

[1]Data are average of 36 observations by sex or 24 observations by line. NS at $P > 0.1$.

[2]Breast weight = weight of pectoralis superficialis + pectoralis profundus.

[3]Breast yield = breast percentage relative to BW.

[4]MFD = myofiber density (number of myofibers per $mm^2$ cross-sectional area of pectoralis muscles).

[5]MFN = total apparent myofiber number of pectoralis muscles.

[6]HBY = commercial broiler strain cross with higher breast yield; NBY = commercial broiler strain cross with normal breast yield; Leghorn = Leghorn-type chickens.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

age (and gender only in the MFN study) as main effects. Pearson correlation coefficients were generated for MFN study to evaluate the correlation of MFN to different economic variables. The Tukey test was used for multiple mean comparison.

## RESULTS AND DISCUSSION

The present study provides a comparison of different chicken genotypes focusing on MFN determined post-hatch in the pectoralis muscle and on myostatin ontogeny during the embryonic period. In the first experiment, the comparison of chicken genotypes based on general performance (BW, breast weight, and breast yield) and pectoralis characteristics at 7, 21, and 35 d are summarized in Tables 1 to 3, respectively. As expected, Leghorns performed poorly compared with the broiler lines, and the HBY line was confirmed to have higher values than NBY for breast weight at 7 d, and for breast yield at 7 and 21 d of age. At 35 d of age, the NBY line showed higher BW ($P < 0.05$), but still lower breast yield than HBY. Breast cross-sectional area was also analyzed in this experiment to calculate MFN. This variable showed a similar pattern to that of breast weight, with HBY showing higher breast area than NBY at 7 and 21 d of age. No difference between the broiler strains was observed for breast area at 35 d of age ($P > 0.05$).

Males showed higher mean values of BW, breast weight, and breast cross-sectional area than females at all ages evaluated, but no sex effect was observed for breast yield ($P > 0.05$).

### *MFD and MFN*

Leghorn-type chickens had higher MFD or more myofibers per square millimeter (i.e., their fibers have a smaller cross-sectional area) compared with broilers, as shown in Figure 2. Comparing genders, females had more myofibers per square millimeter than males at 21 d ($P < 0.05$) (Table 2), but the difference was not significant at 7 d (Table 1). A significant interaction between line and sex ($P < 0.05$) was observed for MFD at 7 and 21 d of age (Figure 3). No gender effect was present for MFD in broilers ($P > 0.05$), but MFD for Leghorns was higher for females than males ($P < 0.05$).

In a previous study (Scheuermann et al., 2003), broiler chicken males were shown to have higher MFD than females at 8 d of age. The observed MFD superiority of females in the present study is probably due to the inclusion of the Leghorn genotype in the analysis. In fact, comparing gender means only in broilers (Figure 3) showed that males did not differ from females in MFD. When the MFD of males and females for the broiler genotypes were compared at 7 and 21 d of age (data not shown), the advantage of males disappeared from 7 to 21 d, although numerically, male superiority in MFD remained. Therefore, compared with females, it is suggested that broiler males have a faster increase in the cross-sectional area of the myofibers (i.e., a higher rate of hypertrophy). At later ages, males would be expected to show lower MFD than females, but this was not determined in the present study.

The effect of selection for growth on increasing the myofiber cross-sectional area has been observed previously (Aberle et al., 1979; Iwamoto et al., 1993; Burke and Henry, 1997) and suggests that the area of the fibers could be a valuable variable in genetic selection programs. In fact, this idea has been considered in pigs. As reviewed by Stickland (1995), pigs selected for large fiber size tend to exhibit larger muscle area, lower total fiber number,

**TABLE 2. BW and characteristics of pectoralis muscles of broiler- and Leghorn-type chickens at 21 d of age[1]**

| | BW (g) | Breast weight[2] (g) | Breast yield[3] (%) | Pectoralis area (mm$^2$) | MFD[4] | MFN[5] |
|---|---|---|---|---|---|---|
| Females | 664.1[b] | 95.7[b] | 13.1 | 1,189.5[b] | 188.6[a] | 174,024 |
| Males | 727.6[a] | 105.3[a] | 13.2 | 1,272.1[a] | 173.2[b] | 182,819 |
| SEM | 11.2 | 2.2 | 0.14 | 23.7 | 4.8 | 6,219 |
| Line[6] | | | | | | |
| HBY | 900.9[b] | 144.1[a] | 16.0[a] | 1,744.2[a] | 120.3[b] | 209,556[a] |
| NBY | 940.3[a] | 134.6[a] | 14.3[b] | 1,615.1[b] | 130.4[b] | 209,909[a] |
| Leghorn | 246.4[b] | 22.8[b] | 9.2[c] | 396.3[c] | 292.1[a] | 115,801[b] |
| SEM | 13.8 | 2.7 | 0.18 | 29.1 | 5.9 | 7,339 |
| CV (%) | 9.2 | 13.0 | 6.5 | 11.5 | 12.3 | 16.5 |
| Line | *** | *** | *** | *** | *** | *** |
| Sex | *** | * | NS | ** | * | NS |
| Sex × line | NS | NS | NS | NS | ** | NS |

[a-c]Means within columns with no common letter differ significantly ($P < 0.05$).

[1]Data are average of 36 observations by sex or 24 observations by line. NS at $P > 0.1$).

[2]Breast weight = weight of pectoralis superficialis + pectoralis profundus.

[3]Breast yield = breast percentage relative to BW.

[4]MFD = myofiber density (number of myofibers per mm$^2$ cross-sectional area of pectoralis muscles).

[5]MFN = total apparent myofiber number of pectoralis muscles.

[6]HBY = commercial broiler strain cross with higher breast yield; NBY = commercial broiler strain cross with normal breast yield; Leghorn = Leghorn-type chickens.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

and poorer meat quality than those selected for smaller fibers. One could extrapolate that the same effect on meat quality could be occurring in broiler chickens, which have larger fiber size than Leghorns, as shown in the present study.

Meat quality concerns with divergently selected chicken have been addressed in previous studies. For example, Schreurs et al. (1995) observed that Leghorns showed higher calpain and lower calpastatin activities than broilers, and more recently, Lonergan et al. (2003) reported greater breast meat shear force for broilers than for Leghorns. Despite such evidence, no problems with tenderness of broiler meat have been reported. However, the effect of increased myofiber cross-sectional area may increase the tendency to pale, soft, exudative meat in chickens. This is becoming an important issue in the poultry industry and objective methods for screening are being evaluated (Galobart et al., 2003).

Broiler genotypes clearly had higher MFN then Leghorns (Tables 1 and 2), probably one of the physiological

**TABLE 3. BW and characteristics of pectoralis muscles of broiler- and Leghorn-type chickens at 35 d of age[1]**

| | BW (g) | Breast weight[2] (g) | Breast yield[3] (%) | Pectoralis area (mm$^2$) |
|---|---|---|---|---|
| Females | 1,393.6[b] | 246.9[b] | 15.9 | 2,193[b] |
| Males | 1,620.7[a] | 294.6[a] | 16.1 | 2,342[a] |
| SEM | 33.5 | 7.5 | 0.22 | 46.5 |
| Line[4] | | | | |
| HBY | 1,926.2[b] | 378.0[a] | 19.2[a] | 3,069[a] |
| NBY | 2,118.8[a] | 385.2[a] | 18.1[b] | 3,017[a] |
| Leghorn | 476.4[c] | 48.2[b] | 10.3[c] | 717[b] |
| SEM | 41.1 | 9.2 | 0.3 | 56.9 |
| CV (%) | 12.2 | 15.2 | 7.6 | 11.2 |
| Line | *** | *** | *** | *** |
| Sex | *** | ** | NS | * |
| Sex × line | NS | NS | NS | NS |

[a-c]Means within columns with no common letter differ significantly ($P < 0.05$).

[1]Data are average of 36 observations by sex or 24 observations by line. NS at $P > 0.1$.

[2]Breast weight = weight of pectoralis superficialis + pectoralis profundus.

[3]Breast yield = breast percentage relative to BW.

[4]HBY = commercial broiler strain cross with higher breast yield; NBY = commercial broiler strain cross with normal breast yield; Leghorn = Leghorn-type chickens.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

reasons for the difference in muscularity. Males had a higher MFN ($P < 0.05$) than females at 7 d but not at 21 d of age. Comparing the broiler genotypes, HBY had a higher MFN ($P < 0.05$) than NBY at 7 d of age. For the line and sex comparisons, however, an interaction between sex and line was observed for MFN at 7 d of age. As shown in Figure 4, the superiority of HBY at 7 d was due to the increased MFN, but only in males. Differentiating the 2 broiler strains based on MFN is complicated by the fact that NBY is also a fast-growing line selected for high breast weight. The relative difference between HBY and NBY strains in breast weight decreased with age (12, 5, and −2%, at 7, 21, and 35 d of age, respectively). However, MFN showed a correlation to breast yield (0.69; $P < 0.001$) (Table 4), indicating that increased MFN may reflect an improved breast yield, and that the difference between HBY and NBY in MFN observed at 7 d of age could be of commercial importance.

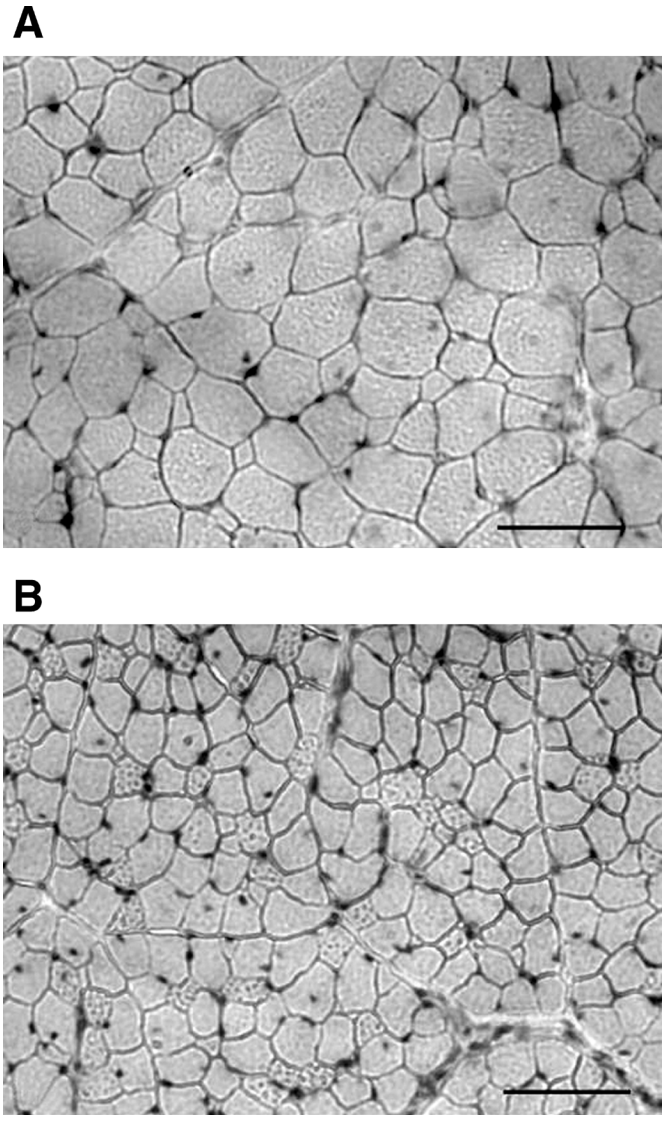


**FIGURE 2.** Cross-sectional slide of pectoralis muscle from broiler- (A) and Leghorn-type (B) chickens. The muscle was sampled at 7 d posthatch, sectioned on a cryostat and hemotoxylin-eosin stained. The pictures are taken at same magnificatin, clearly showing that, at this age, broilers have a larger myofiber cross-sectional area than Leghorns. Scale: 50 $\mu$m.

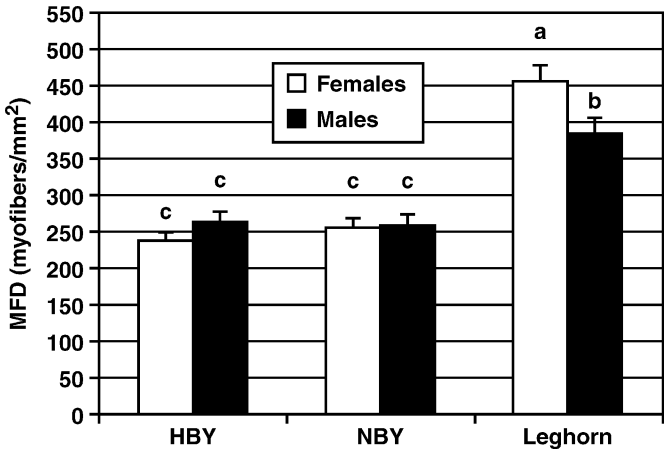


**FIGURE 3.** Line × sex interaction observed for myofiber density (MFD). Data are average of MFD accessed on cross-sections of pectoralis muscle at 7 and 21 d of age. HBY-commercial broiler line with higher breast yield; NBY = normal commercial broiler line; Leghorn = leghorn egg-type chickens. Means with different superscripts differ ($P < 0.05$).

## *Myostatin Ontogeny*

The extreme differences in MFN between broiler- and Leghorn-type chickens demonstrated in this study led us to evaluate the ontogeny of myostatin mRNA expression for these divergently selected chicken lines. Considering that myostatin inhibition has been shown to positively affect the MFN in mice (McPherron et al., 1997), we hypothesized that the chicken genotype difference in MFN could be related to different myostatin expression levels during the embryonic period, when MFN is established (Timson, 1982; Amthor et al., 2002). This was investigated using a semiquantitative RT-PCR method, which was ver-

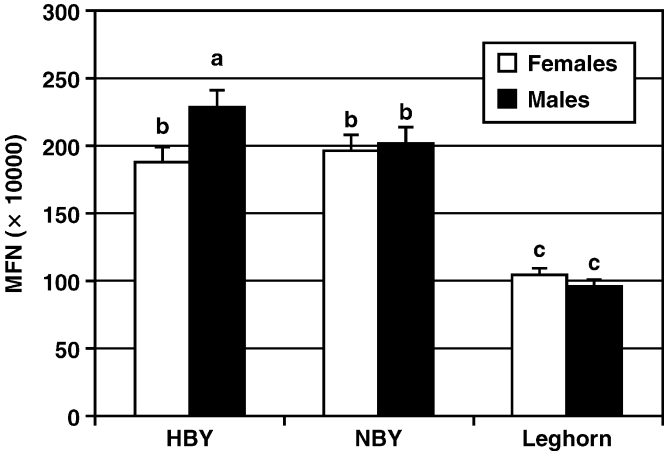


**FIGURE 4.** Line × sex interaction observed for total apparent myofiber number (MFN) counted on slides from pectoralis muscle. MFN values are average from data accessed at 7 and 21 d of age. HBY = commercial broiler line with higher breast yield: NBY = normal commercial broiler line; Leghorn = leghorn egg-type chickens. Means with different superscripts differ ($P < 0.05$).

**TABLE 4. Pearson's correlation coefficients[1]**

| | BW | Breast weight[2] | Breast yield[3] | Breast CSA[4] | MFD[5] |
|---|---|---|---|---|---|
| Breast wt | 0.99 | | | | |
| Breast yield | 0.89 | 0.89 | | | |
| Breast CSA | 0.97 | 0.97 | 0.94 | | |
| MFD | –0.85 | –0.83 | –0.88 | –0.8 | |
| MFN[6] | 0.58 | 0.58 | 0.69 | 0.68 | –0.49 |

[1]The correlation analysis combined data from ages 7, 14, 21, and 35 d (n = 288), except MFD and MFN, which could be correlated to other variables only at 7 and 21 d (n = 144). All significant ($P < 0.001$).

[2]Breast weight = weight of pectoralis superficialis + pectoralis profundus.

[3]Breast yield (%) = breast percentage relative to BW.

[4]Breast CSA = cross-sectional area of total breast muscular area.

[5]MFD = myofiber density (number of myofibers/mm$^2$).

[6]MFN = total apparent myofiber number.

ified in preliminary investigations. The method involved reverse transcription of the same amount of total RNA from each sample, and subsequent PCR amplification of the resulting cDNAs. Primers for a standard gene were also used in similar conditions, but in separate tubes, to avoid any possible primer interaction or limitations of resources due to competition. The ratio of the bands of amplified cDNA to the standard, which were visualized and measured by densitometry after submitting the PCR products to electrophoresis, was used as the index of the relative mRNA level. Three densitometric variables (volume, area, and density) were evaluated in a preliminary study (Figure 5), showing increased densitometric response with the elevation of initial RNA template. The response was linear over a wide range, with reduction in the densitometric increments being observed when the RNA template used was higher than 0.5 $\mu$g/tube. Based on this assay, 0.1 $\mu$g of RNA template was used per RT-PCR tube in the main ontogeny experiment. Although the response was similar for the 3 densitometric variables evaluated, relative density showed the lowest variability (data not shown), and was the choice for the ontogeny study.

Myostatin mRNA expression for broiler- and Leghorn-type chickens, during E1 to E20, is shown in Figure 6. No consistent differences between the genotypes were observed, in agreement with data reported by Mott and Ivarie (2002) comparing broiler- and Leghorn-type chickens at E10 and at 10 d posthatch using Northern blotting. The present study considered a broader range of chicken embryonic development, and used a different methodology to detect the expression of myostatin.

Based on the difference in muscularity between broiler- and Leghorn-type chickens and the implication of myostatin as a negative regulator of myogenesis as shown in myostatin knockout mice (McPherron et al., 1997), we had expected higher myostatin mRNA expression levels in Leghorn-type chickens at an early embryonic age. The embryos in the present study were not staged, and therefore lacked precision in embryonic age. On the other hand, staging embryos based on somite numbers may not have added much in precision, because of the different genotypes used in this study. In quail, strains selected for fast growth rate have delayed somite development (Coutinho et al., 1993). Although no difference in expression was detected at the level of mRNA, it is conceivable that there are differences at other levels of control such as tRNA or folding of the myostatin protein.

Embryonic age effect of myostatin mRNA expression was detected ($P < 0.05$), with multiple mean comparisons

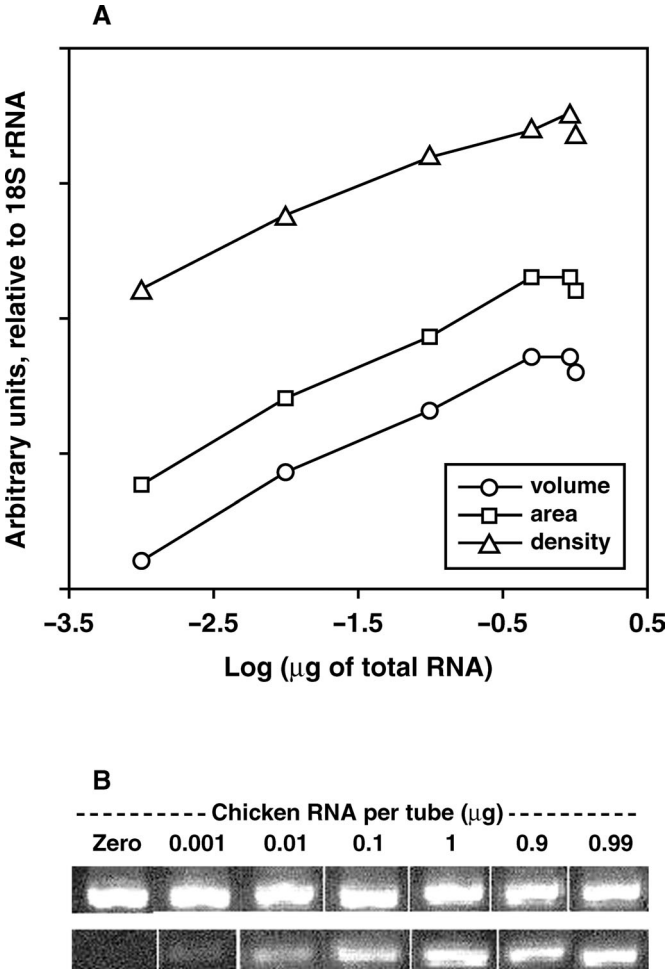


**FIGURE 5.** A) Densitometric response to increased RNA template quantity (log transformed). Three densitometric variables were analyzed: volume, area, and density. B) A representative picture of gel electrophoresis of PCR products. While total RNA in the tube was maintained constant with pig RNA, chicken RNA increased (see Materials and Methods).

showing lower expression levels ($P < 0.05$) around the E3 to E6 period (Figure 6). It is known that myostatin is initially expressed in a central domain of the dermomyotome compartment of developing somites and during the limb bud development when splitting of muscle is underway (Amthor et al., 2002). Because there is evidence that myostatin acts negatively on myogenesis by inhibiting myoblast proliferation (Thomas et al., 2000) and differentiation (Langley et al., 2002), the presence of myostatin at the somite level indicates that a control for myoblast proliferation and differentiation is already in place at early stages of embryonic development. This might explain the high level of myostatin observed early in the present study.

In general, our observations have some similarities to those reported by Kocamis et al. (1999), based on Leghorn-type chicken embryos. According to Miller and Stockdale (1986), chicken primary myoblasts are formed from E4 through E7, and secondaries from E8 through E12. Although lower levels of myostatin expression seem to correspond to the period of primary myoblast formation, the relatively high levels observed around the time for secondary myoblast formation period is intriguing. It was first believed that myostatin expression is restricted to the myogenic lineage (McPherron et al., 1997), but more recently, its expression at early stages of limb bud development was observed in both myogenic and nonmyogenic regions (Amthor et al., 2002). Moreover, Sharma et al. (1999) detected myostatin in heart of the ovine species. If this was also true in chickens, it would certainly increase the complexity of evaluating the expression levels.

The presence of myostatin at later stages, when the muscles are already synthesized, might indicate a chalone-type action of myostatin to control the muscularity considering the space limitations of the in ovo environment. Similar increases in myostatin transcription with aging have also been observed in the postnatal period in mice (Mallidis et al., 1999) but not in humans (Welle et al., 2002).

In conclusion, Leghorns have higher MFD than broilers, but the total MFN in broilers is about twice that of Leghorns. Overall, MFN correlated with BW, breast weight, and breast yield. In addition, high breast yield of broiler strains may partially be due to increased MFN. Based on data generated from unstaged embryos, higher muscularity of broiler chickens relative to Leghorns is not related to lower expression of myostatin at embryonic ages.

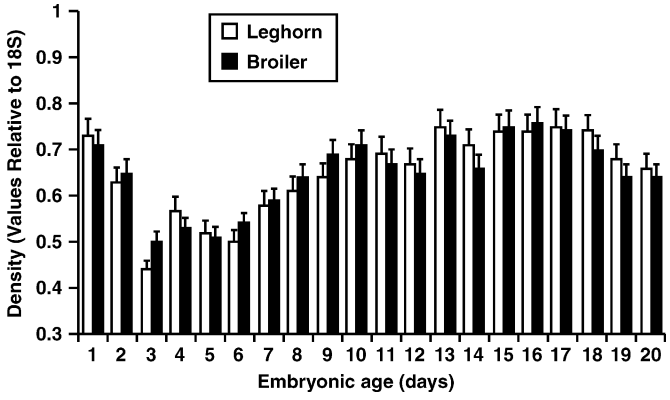


**FIGURE 6.** Ontogeny of mystatin mRNA expression during embryonic period of Leghorn- and broiler-type chickens (average data ± SEM). *Means are significantly different from other groups ($P < 0.05$).

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