Divergent selection for relative breast yield at 4 D posthatch and the effect on embryonic and early posthatch development

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ABSTRACT Genetic selections for growth promotion in poultry have been highly successful in improving growth, yield, and feed conversion in the modern broiler. These selections have focused on the use of hypertrophy, the increase of muscle fiber size to improve growth. Muscle growth however is not limited solely to hypertrophy but is largely attributable to both hypertrophy and hyperplasia, the increase in muscle fiber number. As muscle fiber size has been theorized to reach an eventual physiological limit, it was determined to develop a novel method of selection focusing on hyperplasia. Divergent selection for 4-day relative breast yield (**BY4**) was chosen as it is believed to occur at point at which muscle cell number per gram is maximized and satellite cell activity is higher than later in life. Using a random bred control population, divergent selection was undergone for BY4. The 2 broiler lines divergently selected for BY4 are noted as the high and low BY4 lines, respectively (high 4-day breast yield and low 4-day breast yield). Heritability estimates for selection of 4-day breast percentage in the upward and downward directions were 0.63 and 0.44, respectively. Divergent selection resulted in clear divergence in BY4 and shows promise in utilizing BY4 to promote broiler growth and body composition.

Key words: divergent selection, breast yield, hyperplasia, hypertrophy

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INTRODUCTION

Poultry breeding programs focus on promoting growth, improving feed efficiency, and increased yield (Rauw et al., 1998). Growth-promoting selections are possible because growth traits exhibit moderate to high heritabilities (Emmerson, 1997) and often correlate with increased feed efficiency. Poultry selections have been so successful that in a study comparing a 1957 broiler population to a 1991 broiler strain, modern broiler weights were 3.2 times greater than the 1957 broiler when both were fed a modern diet (Havenstein et al., 1994). Growth selections currently practiced by the industry rely mainly on postnatal growth or an increase of muscle mass resulting from increased muscle fiber size known as hypertrophy (Rehfeldt et al., 2004). Hypertrophy is only one component contributing to muscle mass, as hyperplasia (muscle fiber number) and to a smaller extent extracellular matrix also contribute (Brown and Stickland, 1994). It has been

suggested that the intense focus and duration of selection for muscle size may soon lead to biological limits for muscle fiber size (Mahon, 1999) and may have been responsible for the development of certain muscle myopathies (Bailey et al., 2015).

To continue making selection progress in body weight and yield, novel methods of selection should be considered. To that end, current research has focused on selection for relative breast yield at 4-day (**BY4**) posthatch and is viewed as a new opportunity to promote growth. As the time needed for a broiler to reach market weight decreases, the percentage of lifespan the bird spends in embryogenesis increased (Halevy et al., 2006). This prenatal period is also a time in which almost all increases in muscle fiber number will occur (Rehfeldt et al., 2004).

It has been hypothesized that selection for BY4 will promote growth as it targets an age at which muscle cell number per gram is maximized (0–4 D posthatch) and a point at which satellite cell activity is much greater then later in life (Halevy et al., 2006). Therefore, it may be assumed that the relative breast yield of day 4 chicks relies primarily on the muscle fiber number, as opposed to fiber size. Thus, selection should result in increased fiber number at hatch, with an increase in available satellite cells thus promoting postnatal hypertrophy. In addition, broilers selected for BY4 should exhibit improved growth

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characteristics and improved meat quality. The current study describes the direct response to divergent selection for 4 D breast yield.

MATERIALS AND METHODS

Line Formation

The base population for this study was a random bred line developed in 1997 (Harford et al., 2014). In 2012, divergent selection for BY4 was initiated using a sibling selection protocol similar to that previously described for ascites (Pavlidis et al., 2007) and muscle color (Harford et al., 2014). To establish the research lines, 4 hatches were reared using standard industry practices. Pedigree replacements were derived from hatches 1 and 2 and grown under standard broiler breeder conditions. Hatches 3 and 4 were reared under typical broiler management conditions up to 4 D posthatch. Feed was withdrawn 12 h before euthanasia, and all remaining crop contents were removed after euthanasia. Birds were placed on trays, covered, and refrigerated at 1°C for a minimum of 3 h. After refrigeration, body weights were recorded, as well as weights (g) for bone-in-breast, breast, tenders, keel, and yolk sac. The weight values were then used to calculate the part yields relative to body weight. Sire family selection was used to diverge the random bred control (**RAN**) line into the high 4-day breast yield (HBY4) and low 4-day breast yield (LBY4) lines. Mean percentage breast yield was calculated for the original 24 sire families. The 8 sire families with the greatest percentage breast yield became the HBY4 line, while the 8 sire families with the lowest percentage breast yield became the LBY4 line. Following initial selection, the lines were then considered as closed populations. Each subsequent generation consisted of 4 pedigreed hatches. Hatches 1 and 2 were grown under standard broiler breeder conditions. Birds from hatches 3 and 4 were necropsied for sibling data. Data obtained were used to calculate the dam's estimated breeding values via DMU (Madsen and Jensen, 2000). Based on these estimated breeding values, hatch 1 and 2 progeny from the top 36 dam families were maintained as the selected breeders. Following line development, populations were considered closed.

Embryology

To assess how BY4 selections have impacted embryological development 3 1-week egg collections were incubated. Fertile hatching eggs (N \approx 180 per line) from the HBY4, LBY4, and RAN lines were obtained and incubated under standard conditions and randomly distributed throughout the incubator trays. Collection one was used to assess growth from developmental age 21 to developmental age 24, developmental age being counted from the start of incubation. Collections 2 and 3 were used for samplings from developmental age 15 (E15) to age 20 (E20).

Eggs (N = 30 HBY4, LBY4; N = 20 RAN) were randomly sampled everyday from developmental age 15 to 20. At developmental age 18, eggs not previously selected for sampling were transferred into hatching trays. For developmental ages 21 to 24, 20 chicks were selected for sampling from each of the 3 lines. The procedures used for all collections were identical. Characteristics measured include body, yolk, leg, gastrointestinal tract, and bone-in-breast weight. Supply organ weight was also measured which included the heart, liver, gall bladder, spleen, crop, proventriculus, gizzard, pancreas, large intestine, and all parts of the small intestine. The lungs, although a supply organ, were excluded because of difficulty and inaccuracy of extraction. These studies were conducted in accordance with the recommendations in the guide for the care and use of laboratory animals of the National Institutes of Health, and the protocol was approved by the University of Arkansas Animal Care and Use Committee.

Statistical Analysis

Population parameters were estimated by derivativefree restricted maximum likelihood with a multivariate model using DMU package (Madesn and Jensen, 2000) for body weight, breast, and percentage breast at 4 D of age. Fixed effects accounted for include generation, hatch, and sex for the HBY4 and LBY4 lines. Generational changes in percentage breast yield and additional processing traits were analyzed with line as the main effect by generation and with generation as the main effect by line using the general linear model procedure of SAS 9.3 (SAS Institute Inc., 2001, Cary, NC). Means separations were carried out using Tukey's Honest Significance Difference. Data obtained from the embryological study were analyzed with line as the main effect by developmental age. Trait means separation was performed via the general linear model procedure along with Tukey's Honest Significance Difference using SAS 9.3 (SAS Institute Inc., 2001, Cary, NC). An alpha level of 0.05 was determined to assign significance before testing.

RESULTS

Prehatch Development of BY4 Lines

There was a tendency for higher egg weight for HBY4 as compared with RAN and LBY4. At developmental age 15 and 18 of embryo development, the HBY4 line had a greater egg weight than the RAN and LBY4 lines. However, embryo weight showed no difference at any of the prehatch sampling dates. HBY4 supply organ percentage was greater than the LBY at day 17, no other differences in supply organ percentage were found between BY4 lines at any other age prehatch. Bone-in-breast percentage followed the trend of bone-in-breast weight, where beginning at day 19 and continuing to day 20, the HBY4 line had a greater bone-in-breast percentage than the LBY4. HBY4 bone-in-breast percentage failed to differ from the RAN line at any age prehatch, whereas the RAN bone-in-breast percentage was greater than the LBY4 at day 16 and 20. Leg weight percentage relative to live weight was greater in the HBY4 than the RAN and LBY4 lines at 20 D of age (Table 1).

Early Posthatch Development of the BY4 Lines

At developmental age 21 (i.e. hatch), body weight was lower in the RAN line than either the HBY4 and LBY4 lines, which did not differ (Table 2). There were no differences in body weight at day 22, but from day 23 to 24, the HBY4 has a greater body weight than the LBY4 line. The HBY4 also had a greater body weight than the RAN line at day 23. The RAN and LBY4 did not differ in body weight at either 23 or 24 D. HBY4 line yolk weight was greater than the RAN at days 21, 22, and 23. The LBY4 also expressed greater yolk weight than the RAN at days 22 and 23. There were no differences in yolk weight between the HBY4 and LBY4 at any of the early posthatch sampling dates. At hatch, the RAN line had a greater supply organ percentage than the LBY4 line and continued to have a higher supply organ percentage throughout the early posthatch period, excluding day 23, where no difference in supply organ percentage was shown between any of the BY4 lines. No consistent difference was shown in supply organ percentage between the RAN and HBY4 lines. At day 22, the HBY4 line had a greater percentage bone-inbreast than the LBY4 but did not differ from the RAN line. From day 23 on, the HYB4 had a greater percentage bone-in-breast than either the RAN or LBY4. Neither the RAN nor LBY4 lines were different from each other in percentage bone-in-breast from days 21 to 24. Percentage leg was greater in the RAN than the HBY4 and LBY4 at hatch. By day 24, leg percentage had flipped, with the HBY4 having a greater leg percentage than the RAN and LBY4.

Genetic Parameters

Heritability estimates for 4-day body weight were found to be high in both research lines, greater than 0.4. Breast weight heritability estimates were found to be high for both the LBY4 (0.52) and HBY4 (0.63). Heritability estimates for BY4 were found to be high for both the HBY4 and LBY4 lines, greater than 0.4 (Table 3). Lines expressed clear divergence throughout selection, the line means diverged by 0.33 from generation 1 to 2 and continued to diverge in generations 3 and 4, whereas the average divergence from generation 1 to 4 was 0.19. Generation 4, the HBY4 line had

Table 1. Means¹ \pm SEM for traits measured prehatch from the 4-day relative breast yield lines² after 4 generations of divergent selection.

		4-day breast yield line			
Trait	Developmental ${\rm age}^3$	HBY4	RAN	LBY4	
Egg Wt (g)	15	$53.52 \pm 0.73^{\rm a}$	$51.03 \pm 0.52^{\rm b}$	$51.32 \pm 0.8^{\rm b}$	
	16	51.3 ± 0.60	50.93 ± 0.78	49.84 ± 0.66	
	17	51.3 ± 0.54	50.11 ± 0.64	49.42 ± 0.97	
	18	$50.26 \pm 0.52^{\rm a}$	$49.79 \pm 0.55^{\rm a}$	$48.05 \pm 0.63^{ m b}$	
	19	50.07 ± 0.53	48.25 ± 0.57	48.56 ± 0.88	
	20	48.9 ± 0.61	47.62 ± 0.44	47.93 ± 0.98	
Body Wt (g)	15	17.35 ± 0.19	16.81 ± 0.29	17.1 ± 0.27	
• (0)	16	19.75 ± 0.21	19.86 ± 0.25	19.74 ± 0.23	
	17	23.24 ± 0.25	23.3 ± 0.27	23.32 ± 0.33	
	18	26.73 ± 0.24	26.51 ± 0.32	26.1 ± 0.32	
	19	29.81 ± 0.28	29.5 ± 0.3	30.12 ± 0.45	
	20	32.41 ± 0.33	32.43 ± 0.31	32.43 ± 0.71	
Supply Organ (%)	15	11.06 ± 0.39^{a}	$11.22 \pm 0.58^{\rm a}$	$10.17 \pm 0.44^{\rm a}$	
	16	$9.83 \pm 0.29^{\rm a}$	$10.26 \pm 0.31^{\rm a}$	$10.01 \pm 0.30^{\rm a}$	
	17	$11.48 \pm 0.35^{\rm a}$	$11.04 \pm 0.26^{\rm a,b}$	$10.39 \pm 0.26^{\rm b}$	
	18	$11.19 \pm 0.22^{\rm a}$	$11.50 \pm 0.23^{\rm a}$	$11.04 \pm 0.24^{\rm a}$	
	19	$12.33 \pm 0.27^{\rm a}$	$12.40 \pm 0.27^{\rm a}$	$12.06 \pm 0.27^{\rm a}$	
	20	$13.46 \pm 0.24^{\rm a}$	$13.84 \pm 0.43^{\rm a}$	$13.14 \pm 0.21^{\rm a}$	
Bone in Breast (%)	15	$5.61 \pm 0.17^{\rm a}$	$5.77 \pm 0.20^{\rm a}$	$5.64 \pm 0.15^{\rm a}$	
	16	$5.29 \pm 0.11^{ m a,b}$	$5.65 \pm 0.15^{\rm a}$	$5.21 \pm 0.13^{\rm b}$	
	17	$5.27 \pm 0.09^{\rm a}$	$5.18 \pm 0.10^{\rm a}$	$5.26 \pm 0.08^{\rm a}$	
	18	$5.05 \pm 0.05^{\rm a}$	$5.12 \pm 0.09^{\rm a}$	$5.13 \pm 0.10^{\rm a}$	
	19	$4.89 \pm 0.09^{\rm a}$	$4.76 \pm 0.13^{\rm a,b}$	$4.46 \pm 0.12^{\rm b}$	
	20	$4.80 \pm 0.08^{\rm a}$	$4.70 \pm 0.08^{\rm a}$	$4.39 \pm 0.09^{\rm b}$	
Leg (%)	15	$15.60 \pm 0.51^{\rm a}$	$14.46 \pm 0.66^{\rm a}$	$14.41 \pm 0.68^{\rm a}$	
0 (19)	16	$15.92 \pm 0.40^{\rm a}$	$16.13 \pm 0.63^{\rm a}$	$16.23 \pm 0.36^{\rm a}$	
	17	$17.82 \pm 0.26^{\rm a}$	$17.71 \pm 0.41^{\rm a}$	$18.10 \pm 0.23^{\rm a}$	
	18	$20.21 \pm 0.27^{\rm a}$	$20.36 \pm 0.31^{\rm a}$	$20.25 \pm 0.33^{\rm a}$	
	19	$19.48 \pm 0.37^{\rm a}$	$19.48 \pm 0.28^{\rm a}$	$19.26 \pm 0.26^{\rm a}$	
	20	$20.15 \pm 0.19^{\rm a}$	$19.01 \pm 0.40^{\rm b}$	$19.33 \pm 0.27^{\rm b}$	

Abbrevaitions: HBY4, high 4-day breast yield; LBY4, low 4-day breast yield; RAN, random bred control.

¹Means with no common superscript letter are significantly different between line (P < .05).

 $^2\mathrm{HBY4}$ and LBY4 lines are selected for high and low % breast, respectively.

³Developmental age is started at the time the egg is placed in the incubator.

lines² after 4 generations of divergent selection.

Table 2. Means¹ \pm SEM (g) for traits measured posthatch from the 4-day relative breast yield

		4-day breast yield line			
Trait	Developmental $age^3(D)$	HBY4	RAN	LBY4	
Body Wt (g)	21	$40.5 \pm 0.55^{\rm a}$	$36.33 \pm 1.15^{\rm b}$	$39.15 \pm 0.68^{\rm a}$	
	22	$39.5 \pm 0.87^{\rm a}$	$38.11 \pm 0.67^{\rm a}$	$37.87 \pm 0.61^{\rm a}$	
	23	$47.35 \pm 0.91^{\rm a}$	$44.51 \pm 1.02^{\rm b}$	$43.12 \pm 0.83^{\rm b}$	
	24	$55.84 \pm 1.46^{\rm a}$	$54.31 \pm 1.16^{\rm a,b}$	$50.95 \pm 1.39^{\rm b}$	
Yolk Wt (g)	21	$4.63 \pm 0.16^{\rm a}$	$3.66 \pm 0.21^{\rm b}$	$4.07 \pm 0.25^{\mathrm{a},\mathrm{b}}$	
	22	$2.60 \pm 0.19^{\rm a}$	$1.85 \pm 0.16^{\rm b}$	$2.51 \pm 0.31^{\rm a}$	
	23	$1.00 \pm 0.07^{\rm a}$	$0.77 \pm 0.05^{ m b}$	$0.88 \pm 0.06^{ m a,b}$	
	24	$0.59 \pm 0.04^{\rm a,b}$	$0.55 \pm 0.04^{\rm b}$	$0.79 \pm 0.11^{\rm a}$	
Supply Organ (%)	21	$14.03 \pm 0.43^{\rm a,b}$	$15.08 \pm 0.48^{\rm a}$	$13.7 \pm 0.40^{\rm b}$	
	22	$18.73 \pm 0.96^{\rm b}$	$21.69 \pm 0.69^{\rm a}$	$18.42 \pm 0.50^{\rm b}$	
	23	$27.42 \pm 0.50^{\rm a}$	$28.44 \pm 0.44^{\rm a}$	$27.56 \pm 0.47^{\rm a}$	
	24	$30.84 \pm 0.50^{ m a,b}$	$31.75 \pm 0.38^{\rm a}$	$30.09 \pm 0.55^{ m b}$	
Bone-in-Breast (%)	21	$3.96 \pm 0.10^{\rm a}$	$3.86 \pm 0.13^{\rm a}$	$3.83 \pm 0.11^{\rm a}$	
	22	$3.87 \pm 0.12^{\rm a}$	$3.70 \pm 0.09^{ m a,b}$	$3.58\pm0.08^{ m b}$	
	23	$4.51 \pm 0.07^{\rm a}$	$4.02 \pm 0.09^{\rm b}$	$3.91 \pm 0.08^{\rm b}$	
	24	$5.38 \pm 0.16^{\rm a}$	$4.69 \pm 0.12^{\rm b}$	$4.47 \pm 0.14^{\rm b}$	
Leg(%)	21	$14.18 \pm 0.19^{\rm b}$	$15.35 \pm 0.34^{\rm a}$	$14.42 \pm 0.31^{\rm b}$	
	22	$14.74 \pm 0.27^{\rm a}$	$13.90 \pm 0.40^{\rm a}$	$14.51 \pm 0.27^{\rm a}$	
	23	$14.05 \pm 0.22^{\rm a}$	$13.89 \pm 0.24^{\rm a}$	$13.83 \pm 0.17^{\rm a}$	
	24	$14.07 \pm 0.21^{\rm a}$	$13.33 \pm 0.23^{\rm b}$	$13.50 \pm 0.18^{\rm b}$	

Abbrevaitions: HBY4, high 4-day breast yield; LBY4, low 4-day breast yield; RAN, random bred control.

¹Means with no common superscript letter are significantly different between line (P < .05).

 $^{2}\mathrm{HBY4}$ and LBY4 lines are selected for high and low % breast, respectively.

 $^3\mathrm{Developmental}$ age is started at the time the egg is placed in the incubator. For example, developmental age 24 is a 3 D posthatch chick.

achieved a percentage breast of 2.95, with the LBY4 at 2.21 (Figure 1).

DISCUSSION

The estimated 4-day body weight heritability is consistent with research estimating body heritability between 0.43 to 0.52 in 4-week-old and 6-week-old broilers, respectively (Kuhlers and McDaniel, 1996). The high heritabilities found for breast weight in the LBY4 and HBY4 are consistent with the estimated breast weight heritability of 0.51 found in broilers at 6 wk of age (Le Bihan-Duval, et al., 1999). Heritability estimates for 4-day percentage breast yield were found to be higher than the estimated heritabilities for muscle fiber number calculated by Larzul et al. (1997) and Fiedler et al. (2004). The results are consistent with heritabilities calculated for breast yield

Table 3. Heritabilities, and genetic correlations¹ for body weight and breast traits calculated from the HBY4 and LBY4 lines².

		Line designation					
		HBY4 line		LBY4 line			
Trait	BW	BRST	%BRST	BW	BRST	%BRST	
Body weight Breast ³ % Breast	0.41 0.03 0.06	0.79 0.63 0.01	0.54 0.92 0.63	0.45 0.03 0.04	0.79 0.52 0.01	0.65 0.96 0.44	

Abbreviations: BRST, breast; HBY4, high 4-day breast yield; LBY4, low 4-day breast yield.

¹Heritabilities are on the diagonal line (boldface), genetic correlations are above the diagonal line, and genetic correlation SE are below the diagonal line.

 $^2\mathrm{HBY4}$ and LBY4 are selected for high and low % breast, respectively. $^3\mathrm{Pectoralis}$ major only.

percentage ranging from 0.43 in turkey toms at 14 wk (Case et al., 2012) to 0.55 at 6 wk in broilers (Le Bihan-Duval et al., 2001). Results suggest several possibilities. First, selection for 4-day percentage breast yield may be targeting fiber number, with a higher heritability in avian species than swine. Second, selection may be affecting both fiber number and satellite cell development. Or third, early selection may be shifting the age at which hypertrophy begins, as evidenced by the similar heritability of breast percentage at 6 wk in broilers (Le Bihan-Duval et al., 1999; 2001). Regardless of the exact mode of action, both research lines showed the desired movement in percentage breast yield in generation 2, the first generation after divergence. The HBY4 line increased in breast percentage from generation 1 to 3 with a slight decrease from generation 3 to 4. The LBY4 obtained the lowest breast percentage in generations 2 and 4.

The decrease in BY4 line body weights from generation 1 to 2 were likely because of a change in seasonal conditions, as well as a decrease in breeder age. The HBY4 expressing a greater body weight than the LBY4 from generation 2 onward and the RAN line serving as an intermediate with a greater body weight than the LBY4 were the expected outcomes of divergent selection for BY4. Research has shown that selection for muscle fiber number promotes growth (Fiedler et al., 2004); therefore, it is reasonable to assume that divergent BY4 selection would both promote and obstruct growth.

Egg weight was different at E15 and E18, but these were the only days where differences were detected. However, it appears the HBY4 may have had a greater egg weight, and lack of difference in egg and embryo

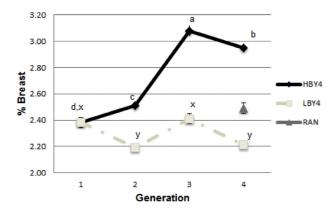


Figure 1. Selection response for percentage breast measures (means \pm SEM) of the BY4¹ lines² over 4 generations of divergent selection. ¹BY4 (4-day relative breast yield). ²HBY4 and LBY4 are selected for high and low 4-day % breast, respectively. ^{a-d}Means for a trait across generations within the HBY line are different (P < 0.05). ^{x-2}Means for a trait across generations within the LBY4 line are different (P < 0.05). Abbreviations: BY4, 4-day relative breast yield; HBY4, high 4-day breast yield; LBY4, low 4-day breast yield; RAN, unselected random bred control.

weight may be because of sampling error, but there is a potential for physiological factors to also play a role. No differences in embryo weight were detected; however, differences were detected in embryo composition as evidenced by differences in supply organ percentage at E17 and bone-in-breast percentages at E16, 19, and 20. The difference in supply organ percentage between the HBY4 and LBY4 at E17 may be demonstrative of the HBY4 line preparing for rapid early growth by having an improved supply system needed to promote growth and muscle development (Siegel, 2002). The lack of difference in supply organ percentage past this point is presumably because of the emergence of differences in bonein-breast percentage between the HBY4 and LBY4, made possible by the increased supply organ percentage at E17.

Embryonically, it has been shown that E16-18 are a key period in satellite cell development (Hartley et al., 1992). As the HBY4 line showed no difference in bonein-breast before E19, it is reasonable to suggest that increased satellite cell proliferation contributed to an increase in bone-in-breast weights. Furthermore, the HBY4 leg percentage was not negatively impacted by increased bone-in-breast, which would be expected as resources are finite, and increased resource devotion to breast yield should have drawn resources needed for concurrent leg development (Siegel et al., 2009), suggesting that increased satellite cell proliferation increased the resource pool available for growth to the HBY4 embryo. The HBY4 lines supply organ weight, and percentages also support the idea that increased satellite cell proliferation resulted in improved bone-in-breast yields, as traditionally selection for increased growth results in decreased supply organ percentage (Deeb and Lamont, 2002).

Muscle fiber number has been shown to be fixed by the time of hatch (Smith, 1963), but research has also demonstrated that the maximum number of muscle cells

per gram of tissue can be found up to 3 D of age dropping significantly at 5 D of age (Halevy et al., 2006). This development is almost perfectly mimicked by the BY4 lines. No differences in body weight between lines were shown at developmental age 22, but at day 23, the HBY4 body weight was greater than the LBY4 and RAN lines. The HBY4 continued to outperform the LBY4 at 24 D, and the RAN line became the intermediate. Furthermore, at hatch, there was no difference in percentage bone-in-breast, but by day 23, the HBY4 line began exhibiting a greater bone-in-breast percentage than the other 2 lines. The change in bone-inbreast suggest that BY4 selection altered the muscle fiber number of the HBY4 and LBY4 lines. This potential increase in fiber number may have resulted in a greater body weight in the HBY4 when compared with the LBY4 and a greater bone-in-breast percentage than either the RAN or LBY4 lines. Although muscle fiber number was not assessed after 4 generation of selection for 4-day, future histological analysis may be able to shed light on possible alteration of fiber number through selection.

The brief time period posthatch is also the point of maximum satellite cell activity (Halevy et al., 2000, 2003) and declines rapidly afterwards (Moss et al., 1964; Halevy et al., 2000, 2003; Velleman et al., 2010). While 4 D posthatch does not represent the peak period for satellite cells, it is a point near maximum satellite cell activity and before gains in muscle fiber size have begun to occur rapidly (Halevy et al., 2006). Increased satellite cell proliferation is suggested both by the bone-in-breast percentage exhibited by the HBY4 line and the leg percentage shown in the HBY4 line. Traditionally, increased breast growth has come without proportional increases in leg muscle (Nestor et al., 1985; Lilburn, 1994; Yalcin et al., 2001). While leg percentage did not increase to the degree of bonein-breast, the HBY4 line still exhibited a greater leg percentage than the other 2 lines at day 24. Again, suggesting that selection could have resulted in increased fiber number and or increased satellite cell proliferation providing the necessary resources to allow for growth in both the breast and legs of the HBY4 chick.

Typically, the aforementioned growth boost would have resulted in a decreased ratio of supply to demand organs (Deeb and Lamont, 2002) in the HBY4 line. This was not seen in the current experiment; rather, the LBY4 line had a lesser supply organ percentage compared with the RAN line, whereas the HBY4 line did not differ from the RAN line. Divergent selection may have resulted in additional resources being devoted to supply organs early on, thereby creating the support system the HBY4 line needed to outperform the LBY4 in body weight and bone-in-breast percentage and the RAN in bone-in-breast percentage. Increased resources would allow for better resource allocation diminishing the imbalance between an increasingly greater body mass and a broilers support system (internal organs, vascular systems, and skeletal structure), which has been cited as a primary cause of the reduction in broiler

fitness (Katanbaf et al., 1988; Dunnington and Siegel, 1996; Julian, 2005).

In summary, selection for BY4 is feasible in single trait because of large breast yield variation and a high heritability for percentage breast at 4 D posthatch. The selected BY4 lines exhibited clear divergence in relative breast yield from the original population. Embryonic differences found between the 3 BY4 lines in supply organ and bone-in-breast percentages demonstrate that divergent selection for BY4 was capable of impacting embryonic development. It appears that the increased supply organ percentage at E17 of development of the HBY4 line allows it to gear up for increased early growth, as evidenced by the increased bone-in-breast percentage shown at days 19 and 20 compared with the LBY4 line. Furthermore, this growth does not come at the expense of leg growth as typically shown in traditional selection (Siegel et al., 2009).

From a practical perspective in the broiler breeder industry, this research may prove useful to selection programs if noninvasive means can be derived to determine fiber number. Owing to the value of pedigree birds and the indirect nature of this selection, it cannot be introduced into selection programs in its current format. However, if noninvasive procedures such as CT of DEXA scanning was utilized to determine breast yield or fiber number at a young age, selection for 4-day breast yield could be incorporated into a breeding program. Research has demonstrated that the combination of increased fiber number and moderate fiber size promotes both meat yield and meat quality (Rehfeldt et al., 2004) which, given the ongoing push for yield and the increased emphasis on meat quality from consumers, makes a technique that can positively affect both muscle growth and quality, such as selection for fiber number evermore desirable.

Whether this growth is the result of increased muscle fiber number, increased satellite cell proliferation, a combination of the 2, or an as of yet undetermined cause is not known. Muscle fiber number was not evaluated following the first 4 generations of selection but would have given insight into the overall selection program and the results that were observed. However, following future generations of selections, histological analysis of muscle fiber number, or satellite cell count will be critical in determining the overall impact of selection for 4-day relative breast yield. While the exact mode of action driving the change in relative breast yield is unknown, results show promise in utilizing BY4 selections to promote broiler growth.

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