Laser enrichment affects myogenic gene expression and breast muscle diameter in Ross 708 broilers

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Summary and Implications

Environmental enrichment designed to stimulate activity may reduce the occurrence of the problematic breast muscle myopathy, woody breast (WB). Successful tools to minimize this disease and resulting economic loss to the poultry industry have yet to be established. The current research objectives were to improve broiler breast muscle quality and myogenic growth potential through environmental enrichment. Six hundred Ross 708 broilers were randomly assigned to enrichment (LASER; laser enrichment, or CON; no laser enrichment) for a 49d study. A subset of 100 birds at each timepoint were used for breast muscle outcomes at d42 and d49. Breast width was measured pre-slaughter, then the right breast fillet was scored for WB using a tactile 0-3 scale and weighed at harvest. RNA isolated from 30 breast muscle samples each at d42 and 49 was analyzed using real-time qPCR. Breast muscle diameter was increased in LASER-enriched broilers on d42 and 49 ($P \leq 0.005$) and breast weight was increased by 30g on d42 ($P=0.039$). At d42, 16% more LASER-enriched WB scores were 0 (normal) compared to the CON, and at d49, 12% more LASER-enriched scores were 0. LASER-enriched broiler breast tissue had upregulated expression of muscle regulatory factor 4 at d42 compared to the CON ($P<0.05$). Taken together, these outcomes indicate that laser enrichment-induced exercise can minimize severity of woody breast without reducing growth.

Introduction

Genetic selection for swift growth and a large proportion of breast muscle in hybrid commercial broilers has inadvertently led to welfare and meat quality issues. In the last ten years, the breast muscle myopathy known as woody breast (WB) has been consistently associated with high breast yielding, fast-growing lines and older, heavier birds. Woody breast is a disease of the *pectoralis major* muscle characterized by discolored, inflexible fillets with a prominent caudal ridge. This condition leads to carcass downgrades at slaughter, diminished raw and cooked poultry product quality, and in severe cases, USDA condemnation of affected tissue. The disorder is hallmarked by heavier, thicker breast fillets. In affected muscle, decreased circulatory supply and significant fibrosis with giant, degraded muscle fibers, are consistently observed. A single underlying cause of WB has not yet been identified.

Primarily described as a meat quality concern, WB is likely a cause or product of diminished animal welfare. Currently in the published literature there have been no attempts to mediate woody breast through increased broiler activity. Inducing exercise is of interest from a meat-quality perspective as exercise is well-known to induce phenotypic changes in skeletal muscle including oxidative capacity, capillarity, and even muscle fiber type. Therefore, the research objectives were to measure the impact of voluntary exercise induced by a laser enrichment device on broiler breast muscle width and fillet weight, WB score, and myogenic gene expression of the pectoralis major at slaughter.

Materials and Methods

Live bird procedures were approved by the Iowa State University Institutional Animal Care and Use Committee.

Animals & Housing

600 mixed sex Ross 708 broilers were obtained from Welp Hatchery (Bancroft, IA, U.S.) on day of hatch and transported to the Iowa State University Poultry Research and Teaching Farm for a 7-week experiment. Birds were randomly assigned to 20 pens (1.22 by 2.44 m/pen) of 30 birds divided across two rooms in the barn.

One room held 10 LASER pens (+ enrichment device), and the other held 10 CON pens (no enrichment device), with an anteroom separating the rooms so crossover exposure was not possible. Environmental conditions and management were kept as identical as possible across rooms. Ten cm of wood shavings provided bedding over concrete floor; PVC pipe dividers with mesh walls (1.22 m height) separated pens. Birds were gradually adjusted from 24 h light on d0 (30-40 lux) to 20 h light (20-30 lux) from d8-49. Diets were formulated to Ross 708 guidelines for starter (d0-d14), grower (d14-28), finisher 1 (d28-42) and finisher 2 (d42-49) performance phases. Birds were fed *ad libitum* out of hanging feeders gradually raised to accommodate bird height, and water was provided *ad libitum* from a hanging water line (approximately 8 nipples/pen).

Laser enrichment

Five laser enrichment devices consisting of two independent red 650 nm lasers each were installed to cover two side-by-side pens. The enrichment device held lasers within a 20.5 by 20.5 cm metal box with glass front mounted above two pens (2.4 m height). Lasers projected in random patterns onto the pen floor for 4-min "laser periods" at: 05:30, 11:30, 17:30, and 23:30 daily for the 7-week trial. Barn and research staff did not enter either room of the barn during the four hours daily containing the laser periods.

Breast width and fillet weight

On d42, and 49, a subset of 50 birds/timepoint (n=100 total birds) were measured for breast width. The width of the pectoralis major muscle from wing to wing was collected using a seamstress tape measure. Following euthanasia by carbon dioxide, the right breast fillet was dissected and weighed individually.

Woody breast

Woody breast score was assigned on dissected right fillets using a tactile 0-3 scale (n=100 fillets). Score 0 represented a normal fillet that was flexible throughout, 1 represented a mild WB fillet with hardness through cranial and possibly caudal region that remained flexible in the medial/caudal region, 2 represented a moderate WB fillet that was hard but maintained some flexibility in the medial to caudal region, and 3 represented a severe WB fillet with no flexibility. Increments of 0.5 were used to identify intermediate scores based on the degree of woodiness observed.

Myogenic gene expression

Following WB scoring, a segment from the cranial region of 14 fillets/timepoint (on d42, and 49) was collected and flash frozen in liquid nitrogen. Samples were stored at -80°C. RNA was isolated using the RNeasy Fibrous Tissue Kit (Qiagen, MD, U.S.). Seven breast muscle samples/treatment/timepoint were analyzed. Isolated RNA was DNAse treated and diluted to 50 ng/ul for One-Step SYBR Green quantitative PCR (Qiagen, MD, U.S.), tested against 6 muscle-growth and regulation primers of interest and the ubiquitously expressed chicken housekeeping gene, 28s. Primers included: myoblast determination protein 1 (MyoD); myogenin (MyoG); muscle regulatory factor 4 (MRF4); insulin-like growth factor 1 (IGF1); insulin-like growth factor 2 (IGF2); and myostatin (MSTN). Means are reported as adjusted Ct values: $((40\text{-MeanCt}_{\text{test gene}} + [\text{Median 28s}]\text{-}(\text{MeanCt}_{\text{test gene}}))$.

Statistical analysis

All data were analyzed using SAS software version 9.4. PROC UNIVARIATE was used to assess the distribution of data prior to analysis. Woody breast scores, due to the descriptive nature of the scale used, were analyzed for percent distribution of scores with a Likelihood Ratio Chi-Square P-value reported for prediction of score by treatment and diet. The remaining data were were analyzed using PROC MIXED with the main effect of enrichment. For all measures, a value of $P \leq 0.05$ was considered significant.

Results and Discussion

Breast width and fillet weight

Breast width was increased due to laser enrichment ($P \leq 0.005$) at both d42 and 49. At d42 the increase was 1.55cm and at d49 1.48cm. Breast fillet weight collected post-harvest was increased due to laser enrichment at d42 by 30g (P=0.039) but was not affected at d49 (P=0.161; Table 1).

Woody breast

At the 42d timepoint, scores ranged from 0-1 (normal to mild WB). Moderate to severe WB was not observed under our research conditions at week 6. On d42, a greater percentage of LASER-enriched broiler breast scores fell into the normal category than CON (80% vs. 64% received a score of 0 or no WB. Twelve percent of LASER-enriched broiler breasts and 28% of CON broiler breasts received a normal-mild WB score (0.5). Eight percent of both LASER-enriched and CON broiler breasts were scored 1 (Figure 1A).

By 7 weeks of age, WB scores ranged between 0-2. Consistent with the d42 results, a greater percentage of LASER-enriched broiler breasts were normal (36% LASER vs. 24% CON). This translates to 12% greater normal breasts in the LASER-enriched broilers compared to the CON. In the normal to mild category, indicating hardness beginning in the cranial region of the fillet, 32% of LASER-enriched broiler breasts compared to 28% of CON broiler breasts received the score of 0.5. Twenty four percent of both LASER-enriched broiler and CON breasts were scored 1 (mild WB). No CON bird breasts were scored 1.5, while 4% of LASER-enriched broiler breasts fell into this mild to moderate category. In the moderate WB category (the most advanced woody breast category observed in this study), only 2% of LASER-enriched broiler breasts compared to 24% of CON broiler breasts were scored 2 (Figure 1B). This indicates that 22% less LASER-enriched broiler breasts scored were affected with moderate WB compared to the CON. Scores above 2 indicating severe WB were not observed in the current study. Taken together, these outcomes are interesting because woody breast has been consistently associated with heavier, thicker breast fillets. In the current work, it appears that phenotypically, the breast diameter was increased by laser enrichment, and at d42 breast fillet weight was increased, but woody breast was reduced. Exercise is well-known to increase blood supply, leading to to greater nutrient and oxygen delivery to skeletal muscles, hence, a possible explanation is that exercise stimulated by laser enrichment may have reduced WB severity by stimulating more circulation and overall healthy muscle growth and regulation.

Myogenic gene expression

Molecular qPCR data are presented as Adjusted Ct values, calculated relative to the chicken 28s housekeeping gene. Irrespective of harvest age (6 and 7-week samples analyzed) LASER enrichment upregulated gene expression of MyoG (P=0.026), MRF4 (P<0.005), IGF1 (P=0.032), and MSTN (P=0.05), compared to the CON. Breast tissue was collected from two timepoints, d42 and d49, hence the effect of day of slaughter (age) was analyzed and data are presented as treatment*day LSMeans (Figure 2A-B). Day of slaughter impacted the expression of IGF1 (P<0.005) and trended to impact IGF2 expression (P<0.10), with increased expression observed on d49. Myogenin (MyoG) is a transcription factor important to myogenesis and repair; an increase in this transcription factor could be considered an indicator of muscle homeostasis. Expression of MRF4 is related to myoblast differentiation and hypertrophy (increase in muscle fiber size) with exercise. Insulin-like growth factor 1 expression was also increased in LASER-enriched broiler breast tissue; IGF1 is a growth factor in skeletal muscle that serves to regulate muscle fiber hypertrophy and regeneration. Myostatin is a negative regulator of muscle growth; lack of this protein results in excessive muscle size without corresponding increase in force. As breast muscle diameter was increased in LASER-enriched broilers, the increased MSTN expression observed may be an indicator of healthy regulation of muscle mass. The changes in myogenic growth potential measured through mRNA expression as well as reduction in WB score observed in the current work are likely due to laser-induced exercise. In summary, laser enrichment increased Ross 708 breast muscle size while decreasing WB score severity through week 7, and positively shifted myogenic gene expression in the breast muscle indicating normal muscle growth and regulation as a result of exercise stimulated by the enrichment device.

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Table 1. Breast muscle growth measured on straight run Ross 708 broilers. Breast width was measured on live birds and breast weight was collected on right breast fillets at 6 and 7-wk harvest timepoints (n=100 fillets/timepoint). Values are LSMeans (pooled SEM) with the main effect of enrichment.

Breast Measure	Control	Laser	Pooled SEM	P-Value
Width (cm)				
d42	22.67[b]	24.22[a]	0.319	0.001
d49	25.67[b]	27.15[a]	0.360	0.005
Weight (kg)				
d42	0.26[b]	0.29[a]	0.012	0.039
d49	0.40	0.43	0.015	0.161

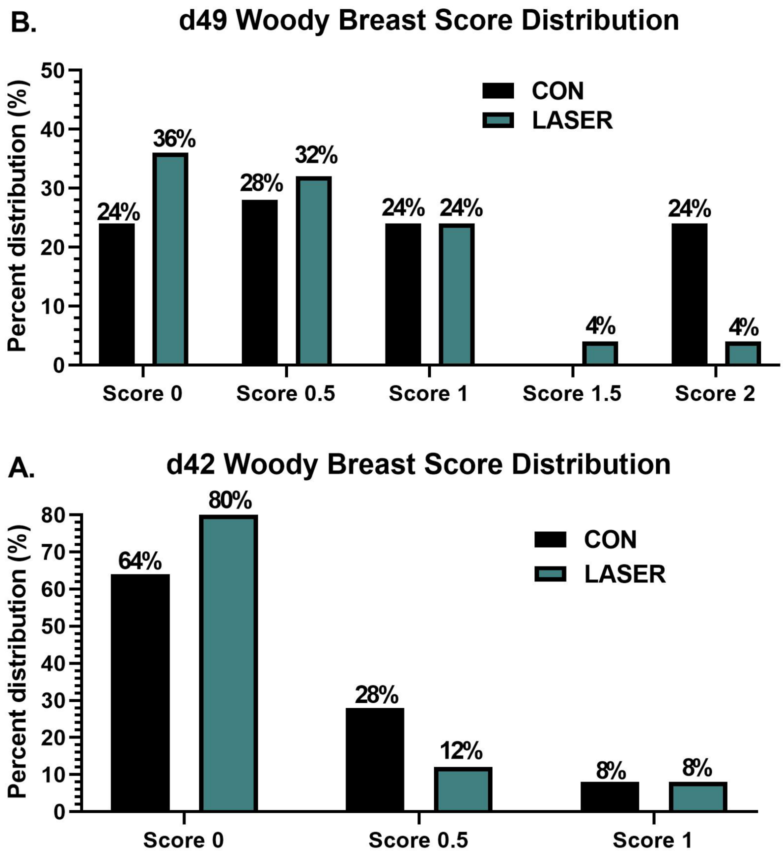

Figure 1. Distribution of Ross 708 broiler woody breast score (n=100 fillets) by enrichment on (A) d42 and (B) d49.

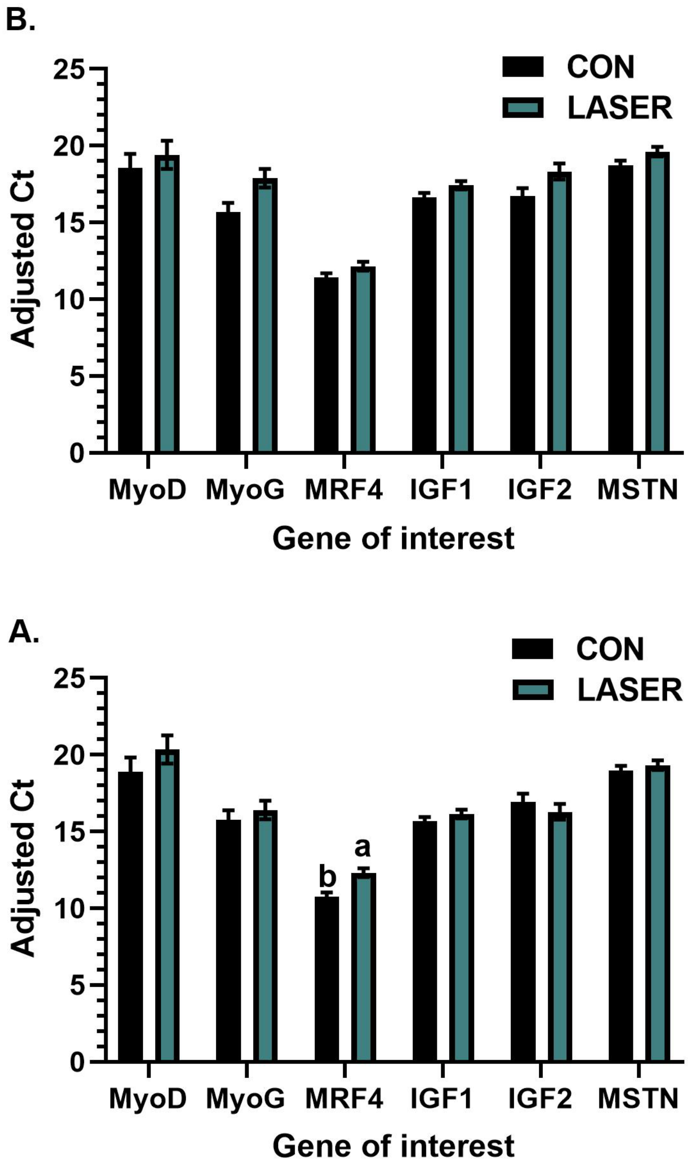

Figure 2. Relative Adjusted Ct values obtained from real-time qPCR using mRNA from cranial portion of Ross 708 broiler *pectoralis major* tissue (n=30 breast muscle samples). LSMeans (±SEM) are presented by main effect of laser enrichment[1] on (A) d42 and (B) d49.

[1]Values lacking common letters within each gene are statistically different (P≤0.05)