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Selection for lean growth efficiency in Duroc pigs influences pork quality

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Abstract
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Comments
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Selection for lean growth efficiency in Duroc pigs influences pork quality[1,2]

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ABSTRACT: A unique line of Duroc pigs was established by intensive selection for increased lean growth efficiency. The objective of this study was to determine the influence of this selection strategy on fresh pork quality traits. Two lines of Duroc pigs originating from the same foundation herd were evaluated. One line was selected for lean growth efficiency over five generations (Select line), and the other was a contemporary line maintained from the foundation herd (Control line). All pigs in the trial tested negative for the halothane gene. Selection for lean growth efficiency resulted in improved lean gain, carcass lean, increased loin eye area, and less overall carcass fat. The Select line had significantly lower subjective firmness scores in longissimus and significantly greater amounts of moisture and protein lost as measurable drip in longissimus, semimembranosus, and semitendinosus. There were no differences in subjective color scores or in Hunter L, a, and b values between lines. No selection line differences were observed in glycolytic potential or ultimate pH. The longissimus and the semitendinosus exhibited significantly lower early postmortem pH values in Select line pigs. Warner-Bratzler shear values were higher for Select line longissimus chops. Degradation of troponin-T was decreased in the Select line longissimus samples. This result suggests that reduced degradation of myofibrillar proteins may be associated with increased moisture and protein lost during storage. This research points out that elimination of the halothane gene will solve some but not all of the genetically influenced pork quality problems faced by the industry. The Select line of pigs appears to be more prone to producing pork that is soft and exudative, indicating a link between soft and exudative pork and some genetic selection strategies may exist. Therefore, it appears that selection for some economically important traits, such as feed efficiency or increased lean growth in the absence of the halothane gene, may compromise pork quality.

Key Words: Meat Quality, Pork, Selection, Tenderness, Water Holding Capacity

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Introduction

One of the prime considerations in the swine industry is to produce the highest quantity of muscle tissue for conversion to meat at the least cost. However, it is becoming increasingly apparent that attention must also be paid to production of uniform, high-quality pork. Reduction in the use of genetic lines carrying the halothane gene (Fujii et al., 1991), associated with pale, soft, and exudative (**PSE**) pork, results in a decreased frequency of PSE product (Lee and Choi, 1999). However, pork quality can be influenced by other factors that are under genetic control. One prominent example is the influence that the Rendement Napole (**RN**$^-$) gene has on low ultimate pH and high drip loss in pork (Monin and Seiller 1985; LeRoy et al., 1990; Milan et al., 2000). This example demonstrates the influence of major genes on pork quality. However, a recent report has suggested that the RN$^-$ gene only partially explains variation in water lost in pork (Bertram et al., 2000). Clearly, the additive effects of a number of other genes also impact pork quality (Hovenier et al., 1992, 1993; Ellis et al., 1996; Cameron et al., 1999).

Kuhlers et al. (1996) established a unique line of Duroc pigs by intensive selection for improved feed conversion and reduced backfat thickness at 105 kg. This Select line of pigs exhibited an increase in lean growth efficiency, decreased backfat, and increased percentage of lean cuts in the pork carcass. This advantage in carcass composition has come at the expense of product quality (Huff-Lonergan et al., 1997). The objectives of

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the study were 1) to characterize the extent of the impact of selection for lean growth efficiency on fresh pork quality and 2) to determine what factors in Select line muscle cause this deterioration in quality. Definition of such factors will provide the background to develop specific selection strategies for improving pork quality from non-stress susceptible animals, to efficiently produce lean pigs that yield high-quality pork.

Materials and Methods

Two lines of Duroc pigs were used in the trial. The halothane genotype of dams and sires in both lines was dominant homozygous stress-negative genotype. The Select line pigs (n = 15) had been selected for improved feed conversion and decreased backfat thickness at 105 kg. The Control line (n = 24) was selected from a contemporary line selected to maintain the performance traits of the original foundation herd. These pigs were obtained from the fifth generation of the Auburn lean-growth efficiency Select and Control lines (Kuhlers et al., 1996). Pigs were slaughtered at 105 kg. Six slaughter groups over 6 wk were used. Pigs were electrically stunned, exsanguinated, scalded (61°C), and eviscerated. A blood sample was taken at exsanguination to screen for the halothane gene stress genotype (Rempel et al., 1993; conducted by Dr. Charles Louis, University of Minnesota). Carcasses were chilled for 24 h in a carcass cooler at 1.8°C.

Temperature and pH of the longissimus (at the last rib) and the semitendinosus were measured at 15, 30, and 45 min and at 24 h postmortem on the right side of the carcass. An Orion pH meter (model 230A) was used with an Orion glass electrode (KNIpHE electrode). A Barnant digital thermometer (Dual J-T-E-K Model) was used to measure temperature. The calibration of the pH meter was corrected for carcass temperature and was checked after each carcass. Carcass data, including average backfat thickness, 10th-rib backfat thickness, and loin eye area, were obtained from the left side of all carcasses at 24 h postmortem (NPPC, 1991). Chops were obtained from the longissimus at the 10th rib interface for evaluation by a trained panel (n = 3) for color, firmness, and marbling using published visual standards (NPPC, 1991). Color (L, a, and b values) of the longissimus and the semitendinosus muscles was measured with the HunterLab Color Difference Meter (D65, 10°), which was calibrated according to manufacturer's directions. Drip loss was determined using 2.54-cm-thick boneless chops (two per animal) from the longissimus (center loin), semitendinosus (center slice), semimembranosus (center slice), and biceps femoris (center slice) muscles. External adipose tissue was removed, taking care to not score the epimysium, and each chop was weighed. Chops were stored in a sealed plastic bag held under atmospheric pressure at 4°C. The liquid lost as drip was removed from each bag and measured after 24, 48, 72, and 96 h of storage. Drip loss was recorded as a percentage of the original weight of the chop. Cumulative and individual time period totals of product lost were recorded. Protein concentration of the drip was determined on drip lost after 96 h (Bradford et al., 1976).

Protein Solubility

Relative protein solubility was determined (Chaudhry et al., 1969; Boles et al., 1992) on fresh longissimus and semitendinosus samples aged 5 d postmortem at 4°C. All extraction, incubation, and centrifugation took place at 4°C. Triplicate samples (2 g each) from each muscle were homogenized in 10 volumes of a low ionic strength buffer (30 m*M* potassium phosphate, pH 7.4) at 4°C. Homogenates were incubated at 4°C with gentle agitation on a rocker for 2 h. Following clarification (1,500 × *g*, 15 min), the supernates were decanted. Pellets were resuspended in low ionic strength buffer, incubated for 2 h, and clarified as above. This step was repeated one more time. Supernates from the three low ionic strength extractions (sarcoplasmic fraction) were pooled. Pellets from the low ionic strength buffer extraction were resuspended in 10 volumes of high ionic strength buffer (500 m*M* potassium phosphate, pH 7.4) and incubated with gentle agitation overnight. Following clarification (1,500 × *g*, 15 min), the supernates were decanted. The pellets were resuspended in high ionic strength buffer, incubated for 2 h, and clarified as above. This step was repeated one more time. The supernates from the three high ionic strength extractions (myofibrillar fraction) were pooled and clarified as above. Soluble protein for both extractions was determined using the Bradford (1976) procedure using premixed reagents (Bio-Rad Laboratories, Hercules, CA).

Warner-Bratzler Shear

Two chops (2.54 cm) per muscle per animal were stored at 4°C for 5 d and were then immediately prepared for Warner-Bratzler shear (**WBS**) force measurements. Chops were cooked in a preheated (177°C) convection oven (Blodget, Model GZL-10). The chops were turned at 5, 10, and, if necessary, 13 min until an internal temperature of 70°C was reached. Temperature of each chop was measured. Three 1.27-cm cores were removed perpendicular to the surface of each chop, for a total of six cores per animal. A single peak shear force measurement was obtained for each core using a Warner-Bratzler Shear instrument (AMSA, 1978; Huff and Parrish, 1993). All cores were sheared perpendicular to the long axis of the core. An average shear force was calculated for each animal.

Calpastatin Activity

Calpastatin activity was determined on fresh semitendinosus and longissimus muscle samples 24 h postmortem. A 10-g sample was extracted in 30 mL of extraction buffer (100 m*M* Tris-HCl, pH 8.3, 10 m*M*

EDTA, 100 mg/L ovomucoid, 2.5 μ*M* E-64, 2 m*M* PMSF). After clarification by centrifugation (40,000 × *g*, 30 min, 4°C), samples were dialyzed overnight against a 50 m*M* Tris-HCl, pH 7.40, 1 m*M* EDTA buffer at 4°C. The dialysate was heated in a preheated water bath (98°C) for 15 min and subsequently cooled in an ice water bath. Coagulated protein was separated by centrifugation as above. Calpastatin activity was determined as described by Koohmaraie et al. (1995b). One unit of calpastatin activity was defined as the ability to inhibit 1 unit of bovine muscle m-calpain activity (Koohmaraie, 1990).

Sample Preparation for Electrophoresis

Longissiumus samples aged 1 and 5 d were prepared according to a whole-muscle protein extraction procedure described by Huff-Lonergan et al. (1996). A frozen section (0.2 g) was removed from the center of the longissimus, knife-minced, added to 5 mL of whole muscle protein extraction buffer (10 m*M* sodium phosphate, pH 7.0, and 2% SDS), and homogenized with a motor-driven Potter-Elvehjem homogenizer. The homogenate was clarified by centrifugation (1,500 × *g*) for 15 min at 20°C. The solubolized protein content of the supernate was determined using the Lowry et al. (1951) method using premixed reagents (Bio-Rad Laboratories, Hercules, CA). Samples were diluted with water to 6.4 mg/mL. One volume of each sample was immediately combined with 0.5 volume sample/buffer tracking dye solution (3 m*M* EDTA, 3% [wt/vol] SDS, 20% [vol/vol] glycerol, 0.003% [wt/vol] pyronin-Y, and 30 m*M* Tris-HCl, pH 8.0) (Wang, 1982) and 0.1 volume of β-mercaptoethanol. The protein concentration of the gel sample was 4 mg/mL. Gel samples were heated to 50°C for 20 min and then frozen at –30°C for subsequent analysis.

SDS-PAGE Electrophoresis and Western Blotting

Fifteen percent polyacrylamide separating gels (acrylamide:bisacrylamide = 100:1 [wt:wt], pH 8.8) were used with 5% polyacrylamide stacking gels (acrylamide:bisacrylamide = 100:1 [wt:wt] pH 6.8) to fractionate whole-muscle proteins. Whole-muscle protein samples (60 μg, longissimus samples prepared as described above) were loaded onto the gels. The same reference (whole muscle extract from porcine longissimus muscle, aged 7 d postmortem) was loaded on each gel. Gels (10 cm wide × 12 cm tall × 1.5 mm thick) were run on a Hoefer SE280 system (Amersham Pharmacia Biotech, Piscataway, NJ) at a constant voltage (120 V) for approximately 2.75 h. Gels were equilibrated in transfer buffer (25 m*M* Tris, 192 m*M* glycine, 2 m*M* EDTA, 15% [vol/vol] methanol). Samples were blotted onto a PVDF membrane at a constant voltage (90 V) for 1.5 h in a Hoefer TE22 transfer tank (Amersham Pharmacia Biotech). The temperature of the transfer buffer was maintained between 4°C and 8°C using a refrigerated circulating water bath.

Posttransfer membranes were incubated for 1 h at 25°C in PBS-Tween (80 m*M* disodium hydrogen orthophosphate, anhydrous, 20 m*M* sodium dihydrogen orthophosphate, 100 m*M* sodium chloride, 0.1 % [vol/vol] polyoxyethylene sorbitan monolaurate [Tween-20]) containing 5% (wt/vol) nonfat dry milk prepared as described by the supplier of the chemiluminescent detection system (Amersham Pharmacia Biotech). Troponin-T blots were incubated with monoclonal anti-troponin-T (JLT-12, Sigma Chemical Co., St. Louis, MO) diluted 1:15,000 in PBS-Tween for 1 h at 25°C. Blots were rinsed three times, 10 min per rinse, in PBS-Tween. Bound primary antibody was labeled with a goat-anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (A2554, Sigma Chemical Co.) diluted 1:5,000 in PBS-Tween, for 1 h at 25°C. Blots were rinsed in PBS-Tween three times, 10 min per rinse, to remove unbound secondary antibody. A chemiluminescent system was used to detect labeled protein bands as described by the supplier (ECL, Amersham Pharmacia Biotech). Relative density of the 30-kDa bands was determined using the Kodak 1D 2.0 software system and a digital camera (Kodak DC120; Eastman Kodak, Rochester, NY). A ratio (30-kDa relative intensity) was calculated (intensity of unknown sample/intensity of reference) to evaluate these values using the internal reference loaded on each gel.

Differences in muscle fiber type were evaluated in 15-min postmortem samples from the longissimus dorsi by separation of myosin isoforms on high porosity SDS-PAGE gels. The procedure used was as described by Talmadge and Roy (1993) with modifications. Muscle samples were extracted in 9 volumes of ice-cold homogenization buffer (250 m*M* sucrose, 100 m*M* KCl, 5 m*M* EDTA, and 20 m*M* Tris, pH 6.8) using a Dounce homogenizer and centrifuged at 1,500 × *g* for 15 min at 4°C. The pellets were then homogenized with a Dounce homogenizer in 25 volumes of whole muscle extraction buffer (2% sodium dodecyl sulfate (SDS) (wt/vol), 10 m*M* sodium phosphate, pH 7.0). Samples were then centrifuged at 1,500 × *g* for 15 min at 20°C. (Huff-Lonergan et al., 1996). Protein concentration of the supernate was determined using the BioRad DC assay (modification of Lowry et al., 1951). Samples were heated in sample buffer (Laemmli, 1970) at 50°C for 20 min at a final protein concentration of 0.125 mg/mL and a total of 2 μg of protein was loaded on each lane of 10 cm × 12 cm × 1.5 mm SDS-PAGE gels. The stacking gels were composed of 30% glycerol, 4% acrylamide-N,N′-methylene-bis-acrylamide (acrylamide-bis) (50:1), 70 m*M* Tris (pH 6.7), 4 m*M* EDTA, and 0.4% SDS. The separating gels were composed of 30% glycerol, 6% acrylamide-bis (50:1), 200 m*M* Tris (pH 8.8), 100 m*M* glycine, and 0.4% SDS. Polymerization of stacking and separating gels was initiated with 0.05% N,N,N′,N′-tetramethylethylenediamine and 0.1% ammonium persulfate. The upper running buffer consisted of 100 m*M* Tris (base), 150 m*M* glycine, 0.1% β-mercaptoethanol, and 0.1% SDS. The lower running buffer consisted of

50 m*M* Tris (base), 75 m*M* glycine, and 1% SDS. Gels and running buffers were cooled to 4°C before use. Gels were run at 125 V for 24 h at 4°C. Bands were visualized by staining with Coomassie blue.

Glycolytic Potential

Longissimus (10th-rib region of right side of carcass) samples were collected and frozen in liquid nitrogen 15 min postmortem, and stored at –80°C until analysis. Glycolytic potential was determined according to the procedures of Monin and Sellier (1985). Briefly, duplicate samples (0.5 g) were extracted in 2.5 mL ice-cold perchloric acid (0.6 *N*) using a motor-driven Potter-Elvehjem homogenizer. Duplicate samples (200 μL) from each homogenate were prepared for glycogen hydrolysis with amyloglucosidase (Sigma Chemical Co.) at 40°C for 120 min. The incubation was stopped with 0.6 *N* perchloric acid and the samples were clarified by centrifugation (1,500 × *g*) for 15 min at 4°C. Clarified samples were used to determine total micromolar glucosyl units (glucose, glucose-6-P, and glucose from glycogen) using the glucose (HK) assay kit (Sigma Chemical Co.). Duplicate samples (2.5 g each) were extracted in 1 *N* perchloric acid for determination of lactate. Lactate (μ*M*) was determined using a kit with premixed reagents per the supplier's recommended procedure (Boehringer Mannheim, Indianapolis, IN). Glycolytic potential was calculated as 2(glycogen + glucose + glucose-6-P) + lactate.

Proximate Analysis

Longissimus samples for proximate analysis were collected at 1 d postmortem and frozen until analysis at the Iowa State University Meat Chemistry Laboratory. Moisture content was determined with an oven-drying method and crude fat was determined by hexane extraction (AOAC, 1990). Protein was determined using a combustion method (AOAC, 1993).

Statistical Analysis

The effect of selection line on parameters measured was determined by analysis of variance using a randomized block design (Steel and Torrie, 1980) with slaughter day as the block (SAS Inst. Inc., Cary, NC).

Results

All pigs in the study tested negative for the presence of the halothane gene. Results summarized in Table 1 demonstrate that selection for lean growth efficiency improved lean gain and carcass composition. The Select line had significantly greater lean gain per day, loin eye area, and percentage lean than the control line. Select line pigs were also leaner than Controls as evidenced by less average backfat and less 10th rib fat.

Temperature and pH of the longissimus and the semitendinosus were measured at 15, 30, 45 min and 24 h.

Table 1. Effect of selection for lean growth efficiency on lean gain and carcass composition

Trait	Select (n = 15)	Control (n = 24)	*P*-value
Lean gain/d, kg	0.292	0.218	<0.01
SE	0.01	0.01	
Loin eye area, cm^2	30.5	26.4	<0.05
SE	1.09	0.94	
10th-rib fat, cm	2.3	3.6	<0.01
SE	0.11	0.09	
Average backfat, cm	2.8	3.7	<0.01
SE	0.09	0.07	
Percentage lean	49.4	41.5	<0.01
SE	0.8	0.7	

The pH of the longissimus from the Select line pigs was significantly lower than that of the Control line longissimus at 15, 30, and 45 min postmortem. The pH of the semitendinosus from the Select line was significantly lower than the semitendinosus from the Control line at 15 min, and 24 h postmortem (Table 2). There were no significant differences in temperature due to selection at any time postmortem for either the longissimus or the semitendinosus muscle (Table 2). Subjective quality scores for the longissimus showed no difference in marbling or color. The Select line longissimus was significantly less firm than the control line longissimus (Figure 1). Hunter L, a, and b values for Control and Select line longissimus and semitendinosus chops were not different (Table 3).

Loin chops from the Select line contained less neutral lipid and more moisture than loin chops from the Control line (Table 4). Select line longissimus had a higher percentage of protein than the Control line. There were no differences in total soluble sarcoplasmic or myofibrillar protein due to selection line. Control line semitendinosus had significantly greater soluble myofibrillar protein than Select line semitendinosus. Selection line had no effect on the calpastatin activity measured at 24 h postmortem in the longissimus. The semitendinosus from the Control line had lower calpastatin activity than did the Select line (Table 4). The longissimus from the Select line pigs had significantly higher shear force values than the loin chops from the Control line pigs. There was no significant difference in shear force values for the semitendinosus chops from the two lines (Table 4).

Selection for lean growth efficiency affected the cumulative 96-h drip loss in chops from the longissimus (Figure 2A), semitendinosus (Figure 2B), and semimembranosus (Figure 2C). At most storage times for each muscle (longissimus, semitendinosus, and semimembranosus), the Select line product had a greater drip loss than did product from the Control line. No significant difference in drip loss between lines was noted at any storage period for the biceps femoris (Figure 2D). Selection line did not affect the protein concentration of the liquid lost as drip. Because a greater

Table 2. Temperature (°C) and pH of longissimus and semitendinosis muscles at 15, 30, and 45 min and 24 h postmortem

Item	Select (n = 15)	Control (n = 24)	*P*-value
Longissimus temperature			
15 min	39.7	40.2	0.75
SE	0.12	0.16	
30 min	39.5	39.9	0.89
SE	0.12	0.17	
45 min	39.1	39.4	0.66
SE	0.15	0.15	
24 h	2.3	2.5	0.14
SE			
Semitendinosus temperature			
15 min	39.4	39.8	0.92
SE	0.16	0.18	
30 min	38.8	39.2	0.21
SE	0.19	0.18	
45 min	38.2	38.3	0.61
SE	0.21	0.22	
24 h	2.8	3.0	0.98
SE	0.23	0.21	
Longissimus pH (measured at the last rib)			
15 min	6.41	6.61	<0.01
SE	0.05	0.04	
30 min	6.25	6.46	<0.01
SE	0.05	0.04	
45 min	6.09	6.35	<0.01
SE	0.06	0.04	
24 h	5.45	5.51	0.26
SE	0.04	0.04	
Semitendinosus pH			
15 min	6.17	6.42	<0.01
SE	0.05	0.04	
30 min	6.07	6.16	0.18
SE	0.05	0.04	
45 min	5.93	6.10	0.07
SE	0.05	0.04	
24 h	5.73	5.88	<0.05
SE	0.05	0.04	

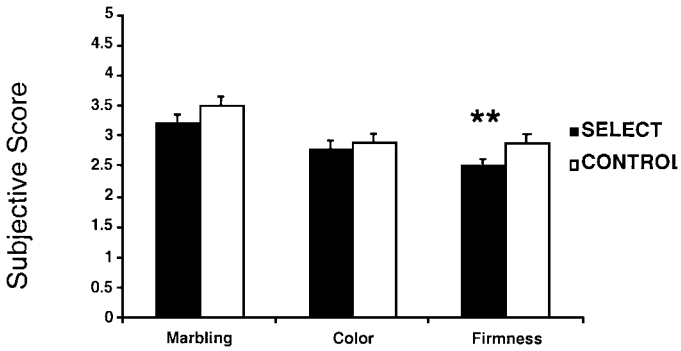

Figure 1. Subjective marbling, color, and firmness scores for Select (n = 15) and Control (n = 24) line longissimus muscle.

Discussion

This current study demonstrated that selection for lean growth efficiency in a closed herd can significantly improve growth and carcass composition traits. The Select line of pigs exhibited a documented increase in lean growth efficiency, decreased backfat, and increased percentage of lean cuts in the pork carcass when compared with the Control line. This type of improvement has been observed with selection for lean growth rate (Cameron and Curran, 1995). Although significant improvements in feed efficiency and carcass composition have been realized in these pigs, meat quality has significantly declined. Loin chops from the Select line pigs had higher Warner-Bratzler Shear values, were less firm, and had poorer water-holding capacity, but were not different from Control line product with regard to color.

It is apparent in this study that selection for lean growth efficiency in these pigs altered the response of muscle to the process of conversion of muscle to meat.

volume of drip was lost, the total protein lost as drip was higher in the Select line longissimus and semitendinosus samples (Table 4).

There was no selection line effect on glycolytic potential in longissimus samples taken at 15 min postmortem (Table 5). The longissimus muscle from the Select line had a significantly more lactate than did the Control line at 15 min postmortem. No differences due to line were detected in the amount of glucosyl units per gram of tissue (glycogen + glucose + glucose-6-P). No differences in specific myosin isoforms were detected between the Select and Control lines (data not shown).

There was no detectable difference in appearance of a 30-kDa troponin-T degradation product between lines in samples aged 1 d (Figure 3). However, there was evidence of more proteolytic activity in Control line samples measured after 5 d postmortem.

Table 3. Mean values for Hunter color values for Select and Control Line longissimus and semitendinosus chops

Item	Select (n = 15)	Control (n = 24)	*P*-value
Longissimus muscle			
Hunter L	43.64	44.47	0.25
SE	0.57	0.43	
Hunter a	3.78	3.87	0.55
SE	0.13	0.11	
Hunter b	6.64	6.84	0.31
SE	0.17	0.13	
Semitendinosus muscle			
Hunter L	42.18	42.09	0.92
SE	0.68	0.62	
Hunter a	5.83	5.76	0.75
SE	0.16	0.14	
Hunter b	7.03	6.95	0.74
SE	0.14	0.15	

Table 4. Mean values for fresh pork composition and quality characteristics in longissimus dorsi and semitendinosus from Select and Control line pigs

Item	Select (n = 15)	Control (n = 24)	*P*-value
Longissimus muscle			
Moisture, % of tissue	72.5	71.7	0.08
SE	0.34	0.25	
Protein, % of tissue	22.3	21.5	<0.01
SE	0.51	0.38	
Lipid, % of tissue	4.50	6.01	<0.05
SE	0.18	0.13	
Protein solubility, mg soluble protein/g tissue			
Sarcoplasmic fraction	70.3	66.3	0.19
SE	2.38	1.82	
Myofibrillar fraction	76.5	74.9	0.74
SE	3.70	2.82	
Calpastatin activity, units/g tissue	1.85	1.72	0.14
SE	0.07	0.05	
WBS, kg d 5 postmortem	3.12	2.62	<0.01
SE	0.10	0.090	
Protein lost as drip over 94 h of storage, mg protein/g product	4.54	3.05	<0.05
SE	0.45	0.44	
Semitendinosus			
Protein solubility, mg soluble protein/g tissue			
Sarcoplasmic fraction	42.1	42.0	0.98
SE	0.78	0.60	
Myofibrillar fraction	35.6	53.6	<0.01
SE	4.21	3.22	
Calpastatin activity, units/g tissue	3.38	2.98	<0.05
SE	0.110	0.097	
WBS, kg	2.25	2.54	0.54
SE	0.149	0.135	
Total protein lost as drip, mg protein/g product	3.47	2.01	<0.05
SE	0.57	0.32	

This alteration has led to significantly lower early-postmortem pH values in both the longissimus (at 15, 30, and 45 min postmortem) and the semitendinosus (at 15 min and 24 h postmortem) from Select line pigs. This is in agreement with the lactate concentration, where Select line longissimus muscle had higher lactate levels at 15 min postmortem. Lactate concentration was significantly negatively correlated with longissimus pH at 15 min postmortem ($r = -0.85$). Differences between the two lines in the rate of pH decline of the longissimus muscle could not be explained by selection line differences in glycolytic potential as glycolytic potential was not significantly different. Because high glycolytic potential is the hallmark of animals carrying the RN^- gene (Monin and Seiler, 1985), neither line demonstrated a phenotype of this genetic condition. Glycogen plus free glucose plus glucose-6-phosphate content at 15 min postmortem was significantly negatively correlated with 24-h pH ($r = -0.46$). This indicates that, regardless of selection line, a greater glycogen and free-glucose content early postmortem led to a lower ultimate pH. A current hypothesis is that selection for growth traits may cause an increase in the percentage of type IIB muscle fibers (Essén-Gustavsson, 1993). This fiber type switch could impact the rate of postmortem muscle metabolism. Using an SDS-PAGE assay to determine differences in myosin heavy chain isoforms, we did not dectect differences in muscle fiber types due to selection in this study. A more rapid metabolism in the early-postmortem period may cause a slight increase in muscle temperature (Cassens, 1966). No differences in temperature were observed at any time point measured. It is possible, however, that the leaner Select line carcasses were chilled more efficiently than the Control line carcasses, thereby negating any metabolically induced differences in temperature.

A striking observation is the consistent effect of selection for lean growth efficiency on the ability of fresh pork longissimus, semimembranosus, and semitendinosus chops to hold water. It is likely that this effect is a direct result of the selection line differences in postmortem pH decline and lactate production by 15 min postmortem. Lactate content (measured 15 min postmortem) and 45-min-postmortem pH were significantly correlated ($r = 0.310$ and $r = -0.337$, respectively) with drip loss during the first 24-h storage period. In

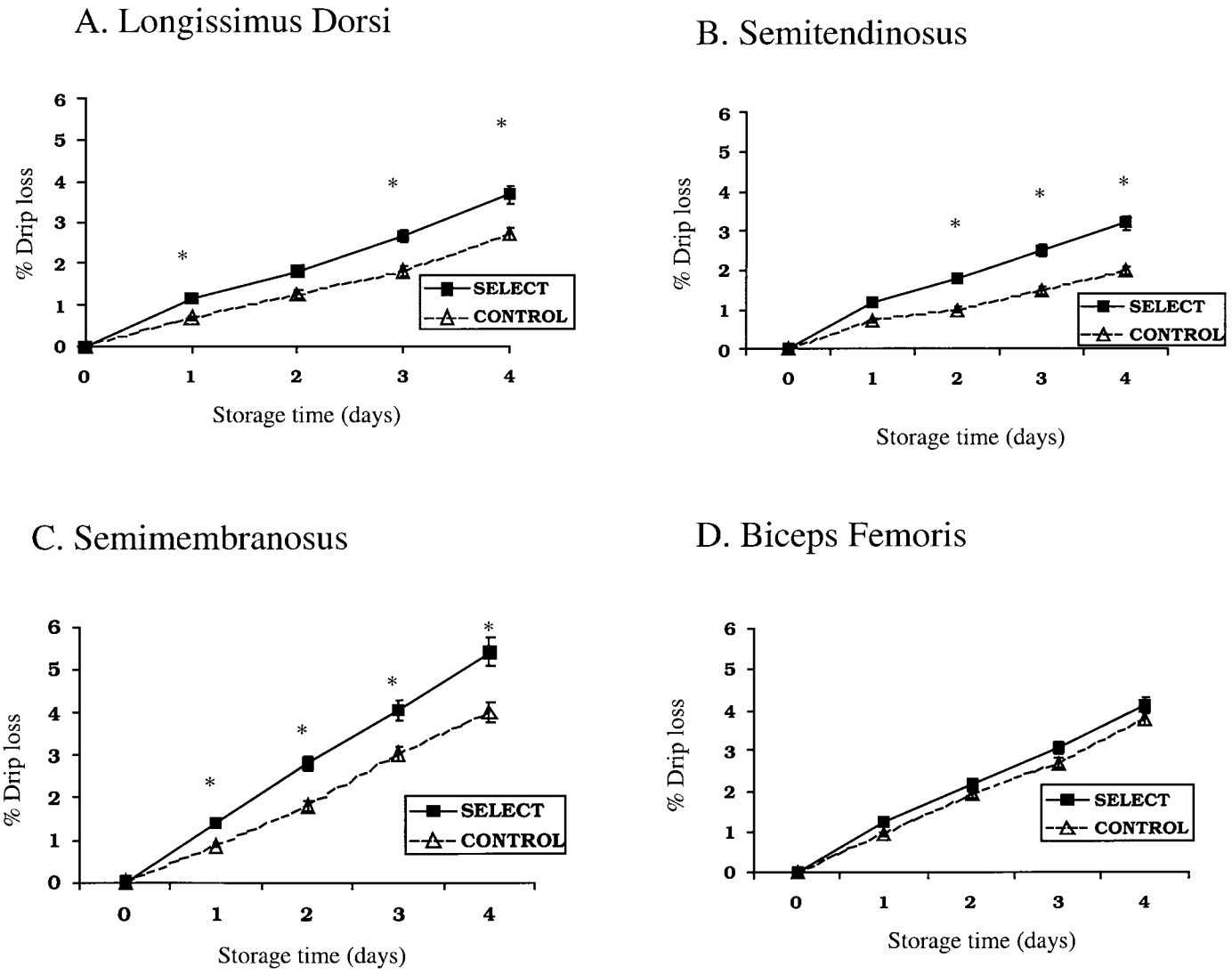

Figure 2. Cumulative drip loss from pork chops during storage at 4°C. A) Longissimus muscle, B) semitendinosus, C) semimembranosus, and D) biceps femoris. An asterisk indicates a significant difference ($P < 0.05$) between Control (n = 24) and Select (n = 15) lines.

contrast, neither of these parameters (lactate at 15 min postmortem and pH at 45 min postmortem) were correlated with percentage product lost as drip during any other time period measured. Conversely, ultimate pH was not significantly correlated with drip loss during the first 24-h period but was negatively correlated with drip loss during the second and third periods evaluated (r = –0.356 and r = –0.400, respectively). These observa-

Table 5. Mean values for glycolytic potential, lactate, and glucosyl units in longissimus dorsi of Select and Control line pigs at 15 min postmortem

Parameter	Select Line (n = 15)	Control Line (n = 24)	*P*-value
Glycogen + glucose + glucose-6-P, μmol glucosyl units/g tissue	29.49	31.60	0.68
SE	2.0	1.7	
Lactate, μmol lactate/g tissue	42.26	29.64	<0.01
SE	2.1	1.7	
GLycolytic potential 2(glycogen + glucose + glucose-6-P) + lactate	101.24	92.84	0.11
SE	3.1	2.6	

tions suggest that water may be lost from different locations or "pools" during postmortem storage and water lost from these pools may be sensitive to different mechanisms. Alternatively, lower pH during the early-postmortem period may cause a shift of intracellular water toward extracellular water, thus accelerating water loss during storage (Offer and Cousins, 1992).

Water-holding capacity may be influenced by proteolysis of myofibrillar protein. Proteolytic disruption at or near the Z-line of the sarcomere has been hypothesized

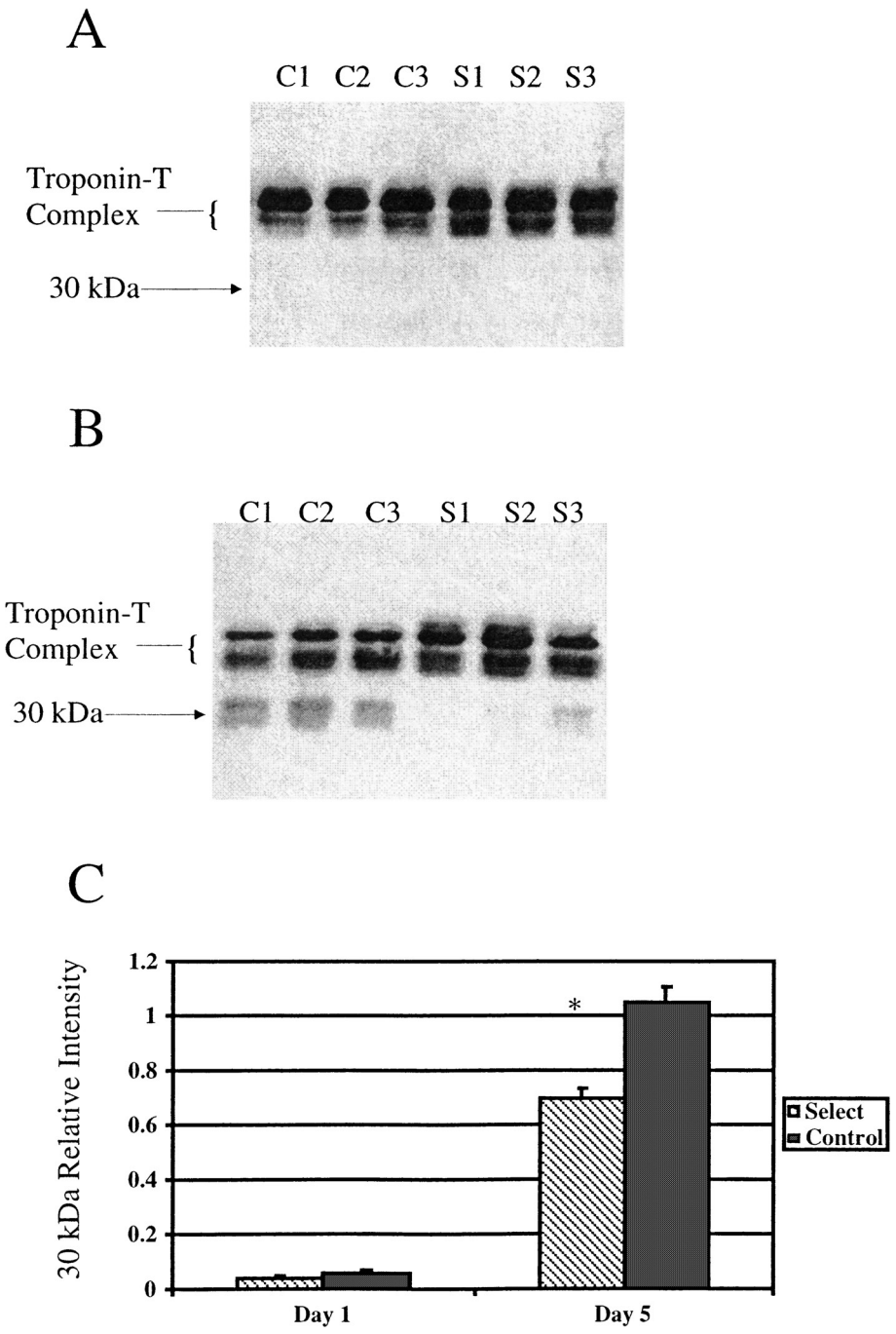

Figure 3. A) Western blot of whole muscle extracts of longissimus muscle chops aged 1 d postmortem using anti-troponin-T antibody. C1 to C3 are Control line samples representative of the Control line based on densitometry results. S1 to S3 are Select line samples representative of the Select line based on densitometry results. B) Western blot of whole muscle extracts of longissimus dorsi chops aged 5 d postmortem using anti-troponin-T antibody. C1 to C3 are from the same animals represented in panel A (C1 to C3). S1 to S3 are from the same animals represented in panel B (S1 to S3). C) Densitometry results from all pigs in the study (Select, n = 15; Control, n = 24). 30-kDa relative intensity is a ratio of intensity of the 30 kDa band against an internal reference on each blot. *Indicates a significant difference ($P < 0.05$) between Control and Select lines.

to dictate the extent of myofibrillar swelling (Offer and Trinick, 1983). This hypothesis is consistent with a reported increase in water-holding capacity in aged meat in which the integrity of the Z-line is lost (Hamm, 1986). Morrison et al. (1998) suggested that proteolysis of intermediate filaments (specifically, desmin) minimizes expulsion of water from muscle cells during drip formation by constraining the muscle fiber, thus reducing its ability to swell. Therefore, a measure of proteolysis in the tissue could shed some light on the mechanisms involved in drip loss.

One protein that is very labile during postmortem storage of meat is troponin-T. Although troponin-T is not classified as a structural protein, its degradation has been shown to be indicative of the amount of protein degradation that has occurred (Lonergan et al., 2001). Degradation of troponin-T by μ-calpain results in specific degradation products ranging from 28 to 32 kDa (Huff-Lonergan, 1996). Troponin-T plays a role in regulating the interaction between actin and myosin. It is possible that disruption of troponin-T may have some impact on interfilament spacing and ultimately the amount of water that can be retained by the myofibril. Alternatively, troponin-T degradation may also be indicative of the amount of general proteolysis that has occurred in meat. In this study, degradation of troponin-T was measured by evaluating the presence of one of the most easily documented degradation products, a polypeptide that migrates on SDS-PAGE gels at approximately 30 kDa. It was noted that the 30-kDa polypeptide was significantly more intense in 5-d postmortem longissimus muscle samples from the Control line than in the Select line. This indicates that less degradation of troponin-T had taken place.

The calpain enzymes have been shown to be responsible for a great percentage of myofibrillar and cytoskeletal protein degradation in postmortem muscle (reviewed by Goll et al., 1998). Calpain enzymes degrade proteins responsible for intra- as well as intermyofibrillar integrity (Huff-Lonergan et al., 1996, reviewed by Koohmaraie, 1996; Huff-Lonergan and Lonergan, 1999). Calpastatin, the endogenous inhibitor of the calpain enzymes, is suggested to be one of the primary regulators of calpain activity in postmortem muscle (Koohmaraie et al., 1995a,b; Lonergan et al., 2001), and has often been associated with variations in postmortem proteolysis in muscle tissue (reviewed by Koohmaraie, 1996; Goll et al., 1998; Huff-Lonergan and Lonergan, 1999). Calpastatin activity (measured 24 h postmortem) was positively correlated with drip loss for the first two 24-h periods of storage (24 h, $r = 0.511$; 48 h, $r = 0.559$). A similar significant relationship was observed with cumulative drip loss at each day of storage. Taken together with the observation of less proteolysis of troponin-T in the Select line longissimus chops at d 5, these relationships indicate that proteolysis of myofibrillar protein may improve the capacity of myofibrils and myofibrillar protein to bind water. Further, calpastatin activity, along with the early-postmortem cellular environment, may regulate the activity of calpain enzymes to degrade myofibrillar proteins. These observations extend the results reported by Boles et al. (1992) and Warner et al. (1997), in which slower proteolysis of titin was associated with decreased water-holding capacity.

Longissimus chops from Select line pigs had higher WBS values than controls. One explanation for this result is the observed difference in intramuscular fat. Control line pigs had higher longissimus intramuscular lipid content than Select line pigs. The influence of intramuscular lipid on objective measures of tenderness in pork has been inconsistent in the literature. Candek-Potokar et al. (1998), Blanchard et al. (2000), and Huff-Lonergan et al. (2000) demonstrated significant, negative relationships between intramuscular lipid and objective measures of textural integrity. These relationships are typically not strong. Other reports have reported no relationship between intramuscular lipid and instrumental measures of textural integrity (Hovenier et al., 1993; Jones et al., 1994). Clearly, the potential of intramuscular lipid to affect WBS also depends on other factors that affect textural properties. These other properties include the amount of postmortem proteolysis of myofibrillar and cytoskeletal proteins.

Degradation of cytoskeletal and myofibrillar protein has the potential to influence pork tenderness. Degradation of muscle proteins during postmortem aging has often been reported to be associated with improvement of tenderness in beef (Olson et al., 1977 reviewed by Koohmaraie, 1996; Lonergan et al., 2001) and in some pork cuts (Wheeler et al., 2000). Selection for lean growth efficiency did not influence calpastatin activity measured 24 h postmortem. Further, calpastatin activity was not correlated with WBS measured 5 d postmortem. This does not exclude the possibility that variations in other postmortem environmental conditions may be influencing calpain activity. The Control line did demonstrate greater proteolysis in loin samples aged 5 d, indicating that proteolysis can explain a portion of the variation in pork tenderness. In fact, relative density of the 30-kDa band in samples aged 5 d was significantly negatively correlated with WBS ($r = -0.484$). The results suggest that variation in overall proteolysis can affect the tenderness of pork longissimus chops. The results of these analyses indicate that selection for lean growth efficiency has reduced postmortem proteolysis of myofibrillar protein and increased WBS in longissimus chops after aging 5 d.

Quality and water-holding capacity differences in the semitendinosus due to selection for lean growth efficiency is most likely explained by a more rapid drop in pH and a slightly lower ultimate pH in the Select line. This variation in early postmortem metabolism in the semitendinosus resulted in less soluble protein in the myofibrillar fraction in the Select line. These factors taken together may explain observed selection line dif-

ferences observed in water-holding capacity in the semitendinosus.

Implications

Selection for lean growth efficiency in a line of Duroc pigs resulted in pork that was normal in color, but was softer and more exudative, and that had higher Warner-Bratzler Shear force values than the Control line. Phenotypic characterization of the Duroc Select line indicate that observed poorer pork quality was not due to the RN^- gene. Pigs utilized in this trial did not carry the stress-susceptible halothane gene. Therefore, we may conclude that selection for some economically important traits, such as feed efficiency or increased lean growth, in the absence of the halothane gene or the RN^- gene compromised some pork quality traits. Future work needs to address identifying the exact biological entities responsible for decreased proteolysis, increased drip loss, and increased Warner-Bratzler Shear force values in products from lines of pigs selected for lean growth efficiency. Pinpointing the causative agents involved in these processes will aid in developing selection strategies that will efficiently produce lean, high-quality pork.

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