An examination of muscle fibers, connective tissue and meat quality in pork from low birth weight

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August 2021

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Introduction

Genetic selection strategies that are used in selecting pigs that are likely to have increased litter size, which will ultimately cause a reduced birth weight in the piglets (Milligan et al., 2002). It has been shown that low birth weight (LBW) piglets (0.95kg to 1.3kg) show reduced postnatal growth rates and a lower lean percentage than those of normal birth weight (NBW) at slaughter (Bee, 2004). Muscle fiber numbers are an important indicator of muscle mass (Miller et al., 1975) because fast-growing pig strains tend to have more muscle fibers than the slow-growing counterpart (Miller et al., 1975). Moreover, birth weight in pigs affects postnatal muscle development, as well as fat accretion, and ultimately the meat quality (Bee, 2004). Chemical analysis revealed that LBW piglets' muscle contain lower fat and protein but higher water than their NBW littermates at slaughter (Rehfeldt and Kuhn, 2006). The total number of muscle fibers in a muscle is lower in LBW piglets than NBW piglets because it is fixed prenatal (Wigmore and Stickland, 1983), but are likely to have a larger mean diameter of muscle fibers when they reach slaughter weight. Muscle fiber types, intramuscular fat content, total collagen and collagen heat-solubility differ according to birth weight and are influenced by meat quality (Lebret et al., 1999). From the above discussion, it is hypothesized that LBW piglets require a longer time to reach slaughter weight due to their lower growth rate, and produce less lean meat that is of inferior eating quality pork.

Therefore, the objective of this study was to investigate the influence of piglets' birth weight on overall growth performance, carcass traits and muscle fiber characteristics, collagen characteristics of *Longissimus* muscle and the consequences on meat quality when piglets were fed with different sources of fat.

Methods and Materials

Animals and diets

Shortly after birth, the piglets were weighted and selected as normal birth weight (NBW), or low birth weight (LBW). All the piglets were fed the exact same diet and management for five weeks of age. At 5 weeks of age, normal birth weight (NBW) piglets and low birth weight (LBW) were randomly assigned to either high fat (HF) diet or HF from dairy source (HFHD) and divided into NBW piglets fed a control diet (NBW-C; n = 5), NBW piglets fed a high fat diet (NBW-HF; n = 6), low birth weight (LBW) piglets fed a high fat diet (LBW-HF; n = 8), and LBW piglets fed a high fat dairy diet (LBW-HFHD; n = 5). Piglets were monitored from week 5 on with weekly check-ins and weigh-ins for a 7-weeks feeding period.

Slaughter and carcass characteristics

At 12 weeks of age, the pigs were euthanized and slaughtered. They were bled, then a blow torch was used to burn away any hairs. The carcasses were washed under cool water and weighted to get a hot carcass weight. The carcass was then placed in a cooler with a bag around it and allowed to cool for 24 hours. The carcass length was measured from the first cervical vertebrae to the base of the tail, before the carcass was butchered for other meat quality parameters, which were all weighted and the weights recorded. The pork loin rib end (thoracic region) from the right side of the carcass was removed and the breakdown of the loin eye muscle for meat quality parameters was described in Figure 1.

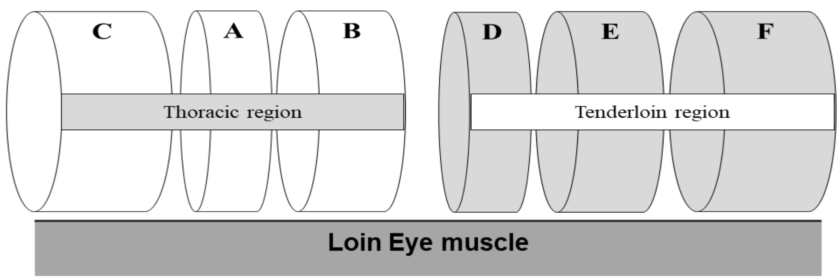

Figure 1: Breakdown of loin eye muscle from right side of the pork carcasses for meat quality characteristics determination at 24 h (thoracic region: A, B, C). One chop/steak (2.54 cm) (A) was used for meat color (subjective and objective), subjective marbling score and texture, subcutaneous fat depth and loin muscle depth to calculate *longissimus* muscle area, and drip loss. Three ribs from the thoracic region (B) were used for cooking and Warner-Bratzler shear force determination. From four ribs from the thoracic region (C) were used for muscle fiber typing, lyophilization, ground and used for determination of proximate components and collagen solubility**.**

Meat quality and muscle fiber properties

Loin meat color was determined after bloom for 20 minutes using a CR-400 chroma meter (Konica Minolta Sensing, Inc., Japan) where lightness (L*), redness (a*), yellowness (b), chroma and hue were measured according to CIE, standard color system (1986).

The ultimate pH was measured with an Accumet AP71 pH meter after calibrated to pH 4.0 and 7.0 standards. The average of three readings was considered for statistical analysis.

Approximately 50-60 g loin meat was suspended in an inflated plastic bag with a metal hook for 24 hours at 4°C and the drip loss was measured as the weight loss during 24 h as a percentage relative to the initial weight. A 2.54 cm thick steaks are cut, weighed and placed in a closed electric grill and cooked until an internal temperature of 71℃, placed in a plastic bag and submerged in ice water to stop cooking.

Cooking loss was determined as- Cooking loss (%) = [(Steak weight before cooking - Steak weight after cooking) / Steak weight before cooking] × 100.

About 100 g of raw meat was cut in small cubes, freeze and freeze-dried for 5-7 days and the moisture content was calculated as- *Moisture* (%) = [(Raw meat weight – Freeze-dried meat weight)/ Raw meat weight] × 100. The crude fat content was measured in freeze dried meat with the Soxtec™ 2050 fat extraction (Foss® Analytical, Hilleroed, Denmark) using petroleum ether as solvent and calculated as-Crude fat (%) = [Intramuscular fat in freeze-dried meat (%) × dry matter (%) in freeze dry meat] / 100. The ash content was measured in freeze dried meat by incinerating in a furnace at 490℃ for 24 h and the ash content was calculated as percentage on raw meat. Crude protein was determined using the AOAC (1990) (Leco Corp., St. Joseph, MI). Freeze-dried meat was combusted and converted to nitrogen. Then nitrogen was converted to protein using a conversion factor of 6.25.

For WBSF, six 1.27 cm diameter cores were removed from the cooked steaks parallel to the direction of the muscle fibers and sheared perpendicular to the muscle fiber direction using a triangular shear blade (Lloyd Instruments LRX plus, AMETEK®, Digital Measurement Metrology Inc. Brampton, ON) at a pre-load force of 2N and a crosshead speed of 200mm per min during shear and then averaged to calculate WBSF for the sample

Muscle fiber types and diameter determination

Longissimus muscle steaks were cut into 1cm^3 cubes, frozen in pre-cooled dry ice acetone. The frozen cubes were used for 10 μm thick serial sections using a cryostat, stained for NADH-TR and Myosin ATPase after pre-incubation in acid (pH = 4.3) and alkaline (pH = 10.5) solution, photographed and the images were then analyzed using ImageJ software to determine and calculate the diameter of different types of muscle fibers (Figure 2).

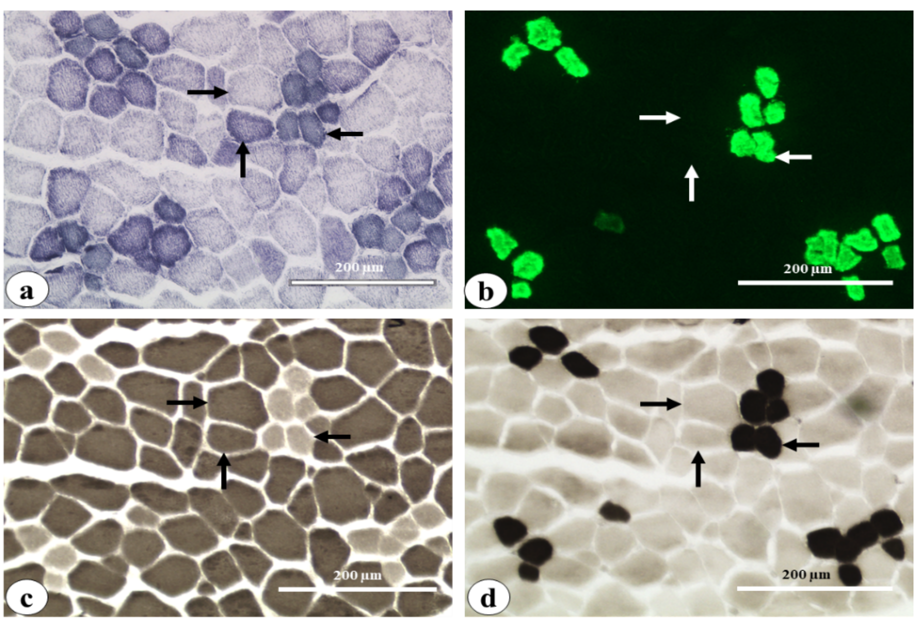

Figure 2: Histochemistry and immunohistochemistry of muscle fibers typing in muscle serial sections from *Longissimus thoracis* of pork (a) NADH-TR; (b) myosin heavy chain type I primary antibody S58 and Alexa fluor 488 green secondary antibody; (c) myosin ATPase activity after alkaline pre-incubation (pH 10.5) and (d) after acid pre-incubation (pH 4.3). Three types of muscle fibers were identified namely fast-twitch-glycolytic (IIB), fast-twitch-oxidative-glycolytic (IIA) and slow-twitch-oxidative (type I) indicated by right arrow, up arrow and left arrow, respectively. Bars = 200μm.

Total, soluble and insoluble collagen determination

Total, soluble and insoluble collagen was determined using freeze dried meat by determining hydroxyproline content following the method Bergman and Loxley (1963).

Statistical Analysis

Data were analyzed by R (version 3.6.1) using the package lessR in one-way analysis of variance (ANOVA) where treatments (birth weight) were considered as the sole source of variation. Where significant main effects were found, pairwise comparisons were carried out by Tukey's Honestly Significant Difference (HSD) test to uncover significant differences between means. All comparisons with $P < 0.05$ were considered statistically significant and with $P > 0.10$ were considered approach significant.

Results

Table 1. Effect of piglet birth weight on carcass characteristics, physical and chemical, and muscle fiber properties of pork at 24 h post-mortem

Parameters	**NBW-C**	**NBW-HF**	**LBW-HF**	**LBW-HFHD**	**P-value**
n	**5**	**6**	**8**	**5**	
Hot carcass weight (kg)	37.08 ± 1.66xy	40.77 ± 1.28x	36.26 ± 2.04xy	33.44 ± 0.89y	0.0615
Subcutaneous fat depth (cm)	1.21 ± 0.14bc	1.45 ± 0.09ab	1.40 ± 0.14ab	0.90 ± 0.04c	**0.0229**
Longissimus muscle area (cm^2)	11.56 ± 2.29	17.18 ± 2.33	15.36 ± 1.51	19.03 ± 1.81	0.1140
Canadian lean yield (CLY, %) **	62.51 ± 0.58ab	61.69 ± 0.34b	62.00 ± 0.72b	64.79 ± 0.34a	**0.0085**
Dressing (%) on hot carcass weight	73.57 ± 1.69	76.17 ± 1.30	72.37 ± 2.56	73.28 ± 0.75	0.5592
Lightness (L*)	50.17 ± 0.61	53.41 ± 1.41	52.12 ± 0.70	52.83 ± 0.50	0.1311
Redness (a*)	8.14 ± 0.32	8.32 ± 0.27	8.25 ± 0.38	8.08 ± 0.31	0.9649
Yellowness (b*)	0.95 ± 0.14b	1.54 ± 0.49ab	1.53 ± 0.27ab	2.52 ± 0.38a	0.0587
Chroma	8.21 ± 0.31	8.56 ± 0.31	8.44 ± 0.42	8.42 ± 0.29	0.9331
Hue	6.59 ± 1.04	10.34 ± 3.22	9.98 ± 1.58	15.03 ± 2.35	0.1359
Ultimate pH 24h	5.53 ± 0.02	5.52 ± 0.04	5.47 ± 0.05	5.52 ± 0.04	0.6784
Drip loss (%)	1.87 ± 0.44y	4.40 ± 1.10x	3.43 ± 0.52xy	2.54 ± 0.16xy	0.0985
Cooking loss (%)	19.91 ± 2.25	18.02 ± 1.51	18.60 ± 0.78	16.82 ± 0.61	0.5138
Warner-Bratzler shear force (N)	31.86 ± 2.78	25.94 ± 2.76	30.94 ± 2.30	26.55 ± 1.49	0.2595
Crude protein (%)	20.97 ± 0.29	21.12 ± 0.08	21.08 ± 0.19	20.86 ± 0.13	0.7955
Moisture (%)	75.82 ± 0.11a	74.67 ± 0.26b	74.89 ± 0.25b	75.11 ± 0.24ab	**0.0229**
Intramuscular fat (%)	1.59 ± 0.10	2.40 ± 0.28	2.38 ± 0.26	2.51 ± 0.33	0.1213

Ash (%)	1.17 ± 0.01	1.12 ± 0.03	1.16 ± 0.02	1.17 ± 0.02	0.2375
Total collagen (mg/ g raw meat)	3.57 ± 0.18	3.20 ± 0.66	2.72 ± 0.14	2.40 ± 0.17	0.1764
Total insoluble collagen (mg / g raw meat)	2.36 ± 0.24	2.27 ± 0.52	1.86 ± 0.10	1.71 ±0.14	0.3873
Total soluble collagen (mg / g raw meat)	1.21 ± 0.08x	0.93 ± 0.21xy	0.86 ± 0.06xy	0.69 ± 0.06y	0.0673
Collagen solubility (%)	35.24 ± 3.65	28.87 ± 5.01	31.62 ± 1.52	28.63 ± 1.88	0.5035
Type I muscle fibers (%)	10.32 ± 0.61	9.27 ± 1.37	9.66 ± 1.83	6.05 ± 0.99	0.2642
Type IIA muscle fibers (%)	16.40 ± 1.07	19.04 ± 1.16	17.00 ± 1.67	18.03 ± 4.11	0.8436
Type IIB muscle fibers (%)	73.28 ± 1.13	71.69 ± 0.71	73.34 ± 2.53	75.92 ± 3.85	0.6970
Type I muscle fibers diameter (µm)	38.86 ± 0.88ab	37.36 ± 1.58b	43.21 ± 1.83ab	44.86 ± 2.70a	**0.0401**
Type IIA muscle fibers diameter (µm)	38.91 ± 3.64	36.30 ± 1.24	39.38 ± 1.76	40.85 ± 1.70	0.5379
Type IIB muscle fibers diameter (µm)	49.94 ± 2.80	45.85 ± 1.29	51.09 ± 2.46	53.25 ± 1.82	0.1723
Mean muscle fibers diameter (µm)	42.57 ± 2.28b	39.83 ± 1.23b	46.76 ± 2.10ab	51.03 ± 4.32a	**0.0057**

Data represents Mean ± standard error (SE)
Different letters (a, b, c) in the same row are significantly different in different animal groups at the 0.05 level of probability ($P < 0.05$).
Different letters (x, y) in the same row are approached to significantly different in different animal groups at the 0.05 level of probability ($P > 0.05$)
NBW= Normal birth weight; **C**= Control (chow) diet; **HF**= High fat diet; **LBW**= Low birth weight; **HDHF** = High dairy high fat diet. HCW = Hot carcass weight
** Predicted lean yield was calculated using the following equation: **CLY** = (%) = 68.1863 − (0.7833 × fat depth in mm) + (0.0689 × muscle depth in mm) +(0.0080 × fat depth in mm^2) − (0.0002 × muscle depth in mm^2) + (0.0006 × fat depth in mm × muscle depth in mm).

The subcutaneous fat depth was significantly lower in LBW-HFHD than both NBW-HF and LBW-HF but did not differ with NBW-C. This indicates that dairy fat (LBW-HFHD) in diets has an influence on reduction of subcutaneous fat.

There was also a significantly higher Canadian lean yield in LBW-HFHD than NBW_HF and LBW-HF but did not differ with NBW-C. This result indicated that high fat in diet reduced lean yield compared with control diet but dairy source high fat diet showed similar lean yield with control.

The moisture content of meat is significantly higher in NBW-C compared with NBW-HF and LBW-HF but did not differ with LBW-HFHD which indicated that high fat diet has influence to decrease moisture in meat but not the high fat diet from dairy source.

Type 1 muscle fiber diameter was significantly higher in LBW-HFHD than NBW-HF but did not differ with NBW-C and LBW-HF which might be that high fat from dairy source has influence on type I muscle fibers hypertrophy which are dependent on fat for their metabolic function. Mean muscle fiber diameter was found to be significantly higher in LBW-HFHD than NBW-C and NBW-HF but did not differ with LBW-HF and might be due to difference in type I muscle fibers diameter.

Conclusion

The LBW piglets that were fed the high fat dairy were able to compensate for their growth with type I muscle fibers hypertrophy, meaning that they were able to catch up with the NBW piglets. The LBW-HFHD piglets also grew with less back fat, meaning that a high fat dairy diet could help combat the tendency of low birth weight piglets gaining more fat mass than their NBW counterparts, as well as to lean yield because of energy partitioning towards muscle rather than towards subcutaneous fat. Dairy fat appears to be a promising source of fat that can be used in a pig diet to reduce subcutaneous fat and increase lean yield, while still growing at an acceptable rate.

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Acknowledgements

Thank you to Dr. Bimol Roy and Dr. Heather Bruce for allowing me to be a part of their research and for being so helpful throughout the entire program.

Thank you to the Faculty of ALES for supporting me this summer.

Thank you to Mrs. Brandi Lee and Mr. Ryan Wales for writing me reference letters that helped me get into the program.

Thank you to WISEST for allowing me to be a part of this program and meet so many amazing people in the process.