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The Type IIB Fibers and the Vascular Network are the Tissue Responsible of the PSE Expression in the Muscle Semimembranosus and Longissimus Dorsi in Pork

[1]H.P. Ngo, [1]P. Figwer, [1]C. Godfraind, [2]M.T. Poirel and [1]M. Franck
[1]Laboratoire de Zootechnie, Ecole Nationale Vétérinaire de Lyon,
69280 Marcy L'Etoile, France
[2]Department of Neuropathology, Clinique St. Luc, ULB, 1200 Bruxelles, Belgium

Abstract: The objective of this study was to determine the effects of the number of the fiber type and the capillary network on the expression PSE in pork. Samples of muscle triceps brachii, muscle longissimus dorsi, muscle biceps femoris and muscle semimembranosus were taken from pigs exhibiting 1 of the 3 HAL geno-types (NN, Nn, or nn). Histoenzymology and immunohis to chemistry were used to compare the fiber type and the capillary network in these muscles within these different stress susceptibility genotypes. In comparison with the reference muscle (muscle triceps brachii), the combination of a high value of the number of type IIb fibers and a low vascular network showed a primary effect on muscles usually involved during stress.

Key words: PSE, halothane, fiber type, vascular network, meat quality, pork

INTRODUCTION

Pale, Soft and Exudative (PSE) pork is a major industry concern due to its undesirable appearance and limited processing functionality (Franck *et al*., 1999, 2000). Pork quality development is largely governed by the rate and extent of postmortem muscle acidification (Briskey, 1964; Sellier and Monin, 1994), which is related to energy availability and demand in the contractile tissue at or around slaughter. Numerous studies have been undertaken to find the major risk factors. Many of them concluded that pigs carrying the halothane-sensitivity (n) allele at the HAL/RYR1 locus (Monin *et al*., 1998; Sellier, 1998; Franck *et al*., 1999), the RN-allele at the rn locus (Monin *et al*., 1998; Sellier, 1998; Franck *et al*., 2000, 2003), or both, present a higher frequency of this defect than normal animals.

So, other risk factors have been evaluated given the likely multiplicity of interwoven mechanisms of PSE meat development. First, it is essential to under-stand why only some muscles (muscle Longissimus Dorsi (LD) and the Muscle Semimembranosus (SM)) are the first involved. According to Lefaucheur *et al*. (2002, 2003) and the earlier results, it appeared that a high proportion of fibers with a glycolytic metabolism could be partly responsible for the PSE expression within some muscles. Furthermore, a low level of vascular could lead to lactic acid be trapped into the less vascularized muscles, which inducing the acid necrosis. The objective of this study was to evaluate the role of the type IIb fibers and the vascular network in several affected or non affected muscles in the PSE meat condition.

MATERIALS AND METHODS

Animals: Twenty six pigs were used in this study, which purchased from Porc hybride society. They were kept in a house for experimental animals in the Ecole Nationale Vétérinaire de Lyon and handled in accordance with national guidelines. They were slaughtered at 100-120 kg of BW.

These genetic tests were done according to molecular biology methods (Labogena, France). They received a general anesthesia (Zoletil 100, tiletamin, 8 mg kg^{-1} IM and myorelaxation with Nesdonal 1 g, thiopental sodium, 20 mg kg^{-1}) before transport to the room autopsy and subsequent exsanguination.

Sample collection and analyses: Thirty minutes after slaughter, samples were taken from the muscle Triceps Brachii (TB); muscle Longissimus Dorsi (LD); the superficial and deep parts of the muscle Biceps Femoris (BF) and the superficial and deep areas of the proximal and median parts of the muscle Semimembranosus (SM). For each muscle, samples were divided in 2 parts: one part was fixed in formalin and the other was promptly frozen in isopentane, cooled to its freezing point by liquid N, then stored at -80°C.

Corresponding Author: Ngo Hong Phuong, Laboratoire de Zootechnie, Ecole Nationale Vétérinaire de Lyon, 1 Avenue Bourgelat, 69280 Marcy L'Etoile, France

Histology: Conventional Hematoxylin-Eosin (HE) (Masson's method) and a Periodic Acid-Schiff's (PAS) staining (Mac Manus method) were performed.

Histoenzymology: Transverse serial sections, 7 mm thick, were cut in a cryostat at -20°C. The conventional myofibrillar ATPase staining after preincubation at pH 4.35 allowed the distinction of 3 fiber types (i.e., black: type I; unstained: type IIa and gray: type IIb).

Immunohisto-chemistry: Immunohisto-chemistry was performed on serial, 7 μm thick sections. The sections were briefiy incubated for 2 h with the smooth muscle α-actin antibody (DAKO, Glostrup, Denmark) and the specific binding was revealed by the avidinbiotin-peroxidase complex (Vectastain ABC kit; Vector Laboratories, Bulingame, CA) method. Capillary pericytes and vascular smooth muscle cells are labeled with this antibody.

Statistical analysis: For statistical analysis, we pooled the results of values obtained with the samples taken from SM (a proximal superficial sample, a proximal deep sample, a median superficial sample and a median deep sample) as well as from BF (a superficial sample and a deep sample). Data were used as variables (number of type I, IIa and IIb fibers; capillaries per area). Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

We try to find the role of the type IIb fibers and the vascular network in the expression of PSE in pork. Because in accordance with Franck *et al.* (1999, 2000, 2002), who showed that only some muscles to be the first involved in this problem such as the muscle Longissimus Dorsi (LD) and the muscle Semimembranosus (SM), while the muscle Triceps Brachialis (TB) corresponds to the most often spared muscle during and after a stress, it was used as a muscle of reference.

Comparison of the 3 groups NN++, Nn++ and nn++, in which 4 muscles LD, SM, BF and TB were studied. The results were showed in the Table 1. Type IIb fiber number showed an increase ($p<0.001$) in the nn++ genotype, but there were not significant difference between the different genotypes in the muscles studied to compare with the muscle of reference (TB) apart from the LD.

A significant reduced capillary network ($p<0.001$) was noticed in LD and SM of the nn genotype to compare with its TB and BF.

The biggest decrease (around 50%) in capillary network was found in the LD of the nn++ group. Apart from a slight decrease in the SM, the total number of fibers was not significant difference among the muscles independently from the genotypes.

The results of type of fiber and the capillary number of 4 muscles LD, SM, BF and TB of the all genotypes together were showed in Table 2. In TB, mean values of fiber typing were 32±17 for type I, 18.6±11 for type IIa and 73±34 for type IIb. The other muscles (LD, BF and SM) harboured a significant increase in the number of type IIb fibers ($p<0.001$).

There were no obvious differences in the size of fiber within 4 muscles studied because there have a similar total fiber number per area. The capillary network value per area of LD, BF and SM were significantly decreased to compare with TB ($p<0.001$), especially in SM (50% less capillary network than in TB: 16.34 and 40.07, respectively).

In this study, we emphasized the obvious decrease in capillary value between the TB, which was mostly spared

Table 1: The type of fiber and the capillary number in the 4 muscles of the 3 groups NN++, Nn++ and nn++

Genotype	Muscles	Type I	Type IIa	Type IIb	Total fibers	Capillary
NN++	LD	12.14±7.330	8.69±8.510	84.60±31.04	105.43±40.48	28.52±16.77
	BF	14.39±11.82	21.40±10.15	105.53±34.97	141.32±32.20	23.65±19.78
	SM	16.03±6.700	5.92±4.920	98.32±22.20	120.27±27.19	14.34±13.54
	TB	44.59±19.56	25.60±12.23	83.67±34.59	145.73±47.01	52.38±16.53
Nn++	LD	13.10±7.620	10.80±7.030	98.76±27.51	122.66±38.40	24.89±10.65
	BF	14.04±10.91	19.28±8.290	112.32±29.46	145.66±29.51	18.27±8.090
	SM	14.46±9.700	8.23±7.210	95.75±27.47	118.45±37.43	9.17±4.580
	TB	30.19±15.08	19.51±8.680	71.65±28.43	121.34±47.22	38.91±12.57
nn++	LD	15.06±9.270	12.32±7.370	126.00±19.22	153.39±24.89	13.78±7.540
	BF	5.85±4.650	20.15±13.36	114.73±19.98	140.75±29.58	32.54±15.49
	SM	9.37±6.110	4.20±5.390	81.26±16.16	94.85±20.48	21.70±12.70
	TB	29.66±16.88	17.61±10.06	101.33±22.74	148.61±30.02	31.31±16.19

Table 2: Type I, IIa and IIb fibers; total fiber number and the capillary number of all genotypes together

Muscles	Type I	Type IIa	Type IIb	Total fibers	Capillary
Longissimus Dorsi (LD)	12.08±7.90	10.20±7.23	100.23±31.75	122.50±40.21	21.32±12.17
Semimembranosus (SM)	13.45±8.26	6.26±6.21	92.24±23.91	111.95±31.82	16.34±12.47
Biceps Femoris (BF)	11.51±10.44	20.26±10.73	110.89±29.01	142.66±30.41	26.40±16.33
Triceps Brachialis (TB)	31.94±17.18	18.56±11.04	73.19±34.53	122.44±46.99	40.07±15.65

and the other 3 muscles, which were usually involved during stress. Follow the earlier reports, the capillary network was directly proportional to the number of type I fibers, which was oxidative fibers in this study, we showed that the TB contained the higher number of type I fibers to compare with the 3 other muscles. This finding justified the choice of the TB as the control muscle, because it was seldom involved.

We noticed an obvious increase in glycolytic type IIb fibers in the 3 other studied muscles. The more the muscle contains glycolytic fibers, the more lactic acid could be increased in the event of stress. Moreover, according to our results, the more the muscle contained glycolytic fibers, the less the capillary network was developed, resulting in a very low level in capillary network exchanges. Consequently, lactic acid might be trapped in these muscles with a reduced capillary network. Capillaries are the only sites of exchanges and for this reason, capillaries were counted in this study and not the arterioles.

Present study also gives new arguments to earlier studies by Sellier and Monin (1994), Larzul *et al*. (1997), Sellier (1998), Lebret *et al*. (1999), Franck *et al*. (1999, 2000, 2002), Le Roy *et al*. (2000) and Laville *et al*. (2005) to elucidate the vulnerability of some muscles with a close correlation to a high level of type IIb fibers and a low level in capillary networks for all the currently studied genotypes.

CONCLUSION

This study has shown for the first time that the combination of a high value of the number of type IIb fibers and a low vascular network showed a primary effect on muscles usually involved during stress (muscle longissimus dorsi, muscle biceps femoris and muscle Semimembranosus) to compare with a reference muscle (the muscle triceps brachii), which is actually not subject to stress-caused problem PSE.

That in turn, would strengthen the need to research ways to minimize stress in the preslaughter period to reduce the risk for stress-susceptible genotypes to develop defects in meat quality.

REFERENCES

Briskey, E.J., 1964. Etiological status and associated studies of pale, soft, exudative porcine musculature. Adv. Food Res., 13: 89-178. PMID: 14283271. http://www.ncbi.nlm.nih.gov/sites/entrez.

Franck, M., G. Benard, X. Fernandez, S. Barbry, P. Durand, T.H. Lagan, G. Monin and C. Legault, 1999. Preliminary observations on the ham destructured: Description of the phenomenon and study of some factors. J. Res. Porc. Fr., 31: 331-338.

Franck, M., G. Monin and C. Legault, 2000. Observations on the ham destructuré: Characterization of the phenomenon by the pH and colour of Semimembranous muscle. J. Rech. Porc. Fr., 32: 345-349.

Franck, M., P. Figwer, M. T. Poirel and X. Pasteur, 2002. Electronarcosis or the CO_2 narcosis method-ideas behind the narcosis stations and their outcome on animal welfare, sanitary safety and the quality of pig meat. Int. Pig Topics, 17: 13-15.

Franck, M., P. Figwer, M. T. Poirel and X. Pasteur, 2003. Incidence of pre-slaughter stress on meat quality of non sensible pigs. Rev. Med. Vet. (Toulouse), 154: 199-204.

Larzul, C., L. Lefaucheur, P. Ecolan, J. Gogue, A. Talmant, P. Sellier, P. Le Roy and G. Monin, 1997. Phenotypic and genetic parame-ters for longissimus muscle fiber characteristics in relation to growth, carcass and meat quality traits in large white pigs. J. Anim. Sci., 75: 3126-3137. PMID: 9419985. http://jas.fass.org/cgi/reprint/75/12/3126.

Laville, E., T. Sayd, V. Santé-Lhoutellier, M. Morzel, R. Labas, M. Franck, C. Chambon and G. Monin, 2005. Characterisation of PSE zones in M. semimembranosus pig muscle. Meat Sci., 70: 167-172.

Lebret, B., P. Le Roy, G. Monin, L. Lefaucheur, J.C. Caritez, A. Talmant, J.M. Elsen and P. Sellier, 1999. Influence of the three RN Genotypes on chemical composition, enzyme activities and myofiber characteristics of porcine skeletal muscle. J. Anim. Sci., 77: 1482-1489. PMID: 10375225. http://www.ncbi.nlm.nih.gov/sites/entrez.

Lefaucheur, L., P. Ecolan, Y.M. Barzic, J. Marion and J. Le Dividich, 2003. Early postnatal food intake alters myofiber matura-tion in pig skeletal muscle. J. Nutr., 133: 140-147.

Lefaucheur, L., P. Ecolan, L. Plantard and N. Gueguen, 2002. New insights into muscle fiber types in the pig. J. Histochem. Cytochem., 50: 719-730.

Le Roy, P., J.M. Elsen, J.C. Caritez, A. Talmant, H. Juin, P. Sellier and G. Monin, 2000. Comparison between the three porcine RN genotypes for growth, carcass composition and meat quality traits. Genet. Sel. Evol., 32: 165-186. PMID: 14736400. http://www.ncbi.nlm.nih.gov/sites/entrez.

Monin, G., P. Seillier and M. Bonneau, 1998. Thirty years of development of the concept of pork quality meat. J. Rech. Porc. Fr., 30: 13-27.

Sellier, P., 1998. Genetics of meat and carcass traits. The Genetics of the Pig. CAB International, Wallingford, Oxon, UK.

Sellier, P. and G. Monin, 1994. Genetics of pig meat quality: A review. J. Muscle Foods, 5: 187-219.