Food Sci. Biotechnol. Vol. 14, No. 5, pp. 628 ~ 633 (2005)

Food Science and Biotechnology

Prediction for Quality Traits of Porcine *Longissimus Dorsi* Muscle Using Histochemical Parameters

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Abstract Muscle fiber characteristics were evaluated for predictability of meat quality traits using 231 crossbred pigs. Muscle pH_{45min}, R-value, and pH_{24hr} were selected to estimate regression equation model of drip loss and lightness, although variances of coefficient estimates could only account for small part of drip loss (about 16.3 to 25.3%) and lightness (about 16.9 to 31.7%). Muscle pH_{24hr} was represented to drip loss and lightness, which explained corresponding 25.3 and 31.7% of estimation in drip loss and lightness, respectively. Area percentage of type IIb fiber significantly contributed to prediction of metabolic rate and meat quality. However, equations predicting meat quality traits based on area percentage of type IIb fiber alone are less useful than ones based on early postmortem parameters. These results suggest estimated model using both metabolic properties of muscle and postmortem metabolic rate could be used for prediction of pork quality traits.

Keywords: metabolic rate, muscle fiber, meat quality, pig

Introduction

Mechanisms controlling pork palatability are often associated with altered postmortem (PM) muscle metabolism such as the rate of glycolysis and the ultimate muscle pH. A high rate of pH decline and a low ultimate pH result in denaturation and diminished quality parameters of the muscle protein (1, 2). Because muscle fibers contain different myosin heavy chains, which are responsible for their different ATPase activity (4), it is possible that the composition of fiber types may be associated with the PM changes in the conversion of muscle into meat and, subsequently, the meat quality (5).

Muscles comprise a mixture of all fiber types (6), and it is generally accepted that the composition of these fiber types influences the metabolic properties of a muscle, which are related to the muscle metabolism in the period around slaughter, and thereby the meat quality (7). This implies that the actual metabolic state of the muscle at slaughter depends on the compositions of the muscle and fiber types. Thus, for practical application of this knowledge to the improvement and control of the meat quality, additional information of the effects of the fiber type characteristics on the PM metabolic rate is necessary. Therefore, in this study the muscle fiber characteristics were examined to predict the PM metabolic rate and meat quality traits.

Materials and Methods

Animals A total of 231 crossbred Duroc × (Yorkshire × Landrace) pigs (149 gilts and 82 castrated male pigs) were evaluated. Pigs were slaughtered at 172.7 ± 1.7 days of age during the winter period. The abattoir used a traditional scalding-singeing process. After electrical-stunning, the carcasses were exsanguinated before scalding in 65°C hot water and weighed after evisceration. The backfat thickness was measured at the 11th and last thoracic vertebrae, and the mean value of these measurements was used as the backfat thickness. The loin-eye area was measured at the level of the last rib.

Histochemical analyses Within 45 min PM, muscle samples for histochemical analysis were taken from the *longissimus* muscle at the 8th thoracic vertebrae. Samples were cut into 0.5 × 0.5 × 1.0 cm pieces, promptly frozen in isopentane cooled by liquid nitrogen, and stored at -80°C prior to use. Serial transverse muscle sections (10 μm) were obtained from each sample with a cryostat (CM1850, Leica, Germany) at -20°C and mounted on glass slides.

The sections were stained for myosin ATPase reactivity according to the method of Brooke and Kaiser (8). Unfixed sections were pre-incubated at room temperature for 5 min in a buffer consisting of 100 mM potassium chloride in 100 mM sodium acetate and adjusted to pH 4.7 with acetic acid (9). After pre-incubation, the sections were subjected to the following steps: washing in four rinses of distilled water; washing for 30 s in a 20 mM glycine buffer (pH 9.4) containing 20 mM $CaCl_2$; incubation at room temperature for 25 min in a freshly prepared medium (40 mM glycine buffer containing 20 mM $CaCl_2$ and 2.5 mM ATP disodium salt, pH 9.4); washing in three 30 s changes of 1% $CaCl_2$; washing in 2% cobalt chloride for 3 min; washing in three changes of distilled water; immersing in 1% yellow ammonium sulfide; washing in several changes of distilled water; mounting in glycerol jelly.

All histochemical samples were examined by an image analysis system. The operational system consisted of an optical microscope equipped with a CCD color camera (IK-642K, Toshiba, Japan), and a standard workstation computer, which controlled all the image analysis system (Image-Pro Plus, Media Cybernetics, L.P., U.S.A.). All portions of the sections without disrupted or freeze-

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Received May 11, 2005; accepted August 23, 2005

damaged tissues were analyzed. About 600 fibers per sample were evaluated and classified as type I, IIa,or IIb by dark, light, and intermediate stainings, respectively.

Postmortem metabolic rate Within 45 min PM, samples from the *longissimus* muscle at the 8th *thoracic vertebrae* were taken and immediately frozen in liquid nitrogen to determine the PM metabolic rate. Early PM muscle pH was measured using a spear type electrode (Model 290A, Orion Research Inc., U.S.A.) at 45 min (pH_{45min}) and 24 hr PM (pH_{24hr}), and the muscle temperature was measured in the center of the muscle using a portable thermometer (Model TES-1300, TES Electrical Electronic Co., Taiwan) directly on the carcass after the muscle samples were removed.

To determine the PM metabolic rate, R-values were measured using the procedure of Calkins *et al.* (10). Briefly, samples placed in 6% perchloric acid were homogenized (Ace Homogenizer AM-8, Nissei Co., Japan) at 5,000 rpm for 90 s and centrifuged (Centrikon T-124, Kontron instruments Co., Switzerland) at 3,000 × g for 10 min at 2°C. The absorbance was measured using a calibrated spectrophotometer (Model Du-64, Beckman Co., U.S.A.). R_{248}, R_{250}, and R_{258} were obtained as the ratios of A_{248}/A_{260}, A_{250}/A_{260}, and A_{258}/A_{250}, respectively.

Meat quality traits Following 24 hr of chilling, *longissimus dorsi* muscle was examined to evaluate the meat quality traits. Drip loss was determined by suspending the muscle samples standardized for the surface area in an inflated plastic bag for 48 hr at 2°C (11). Filter-paper fluid uptake (FFU) was also measured as described by Kauffman *et al.* (12).

The meat color was measured at 45 min and 24 hr PM with a chromameter (CR-300, Minolta Camera Co., Japan) after exposure of the surface to air for 30 min at 2°C. The average of triplicate measurements was recorded, and the results were expressed as L*, a*, and b* values.

Statistical analysis The regression models were established to predict the PM metabolic rate, the protein solubility and the pork quality traits using REG procedure of SAS program. The early PM and histochemical parameters were used as the independent variables. All possible combinations of independent variables were added or deleted sequentially from the models until the R^2 values were obtained from the prediction equations.

Results and Discussion

Postmortem metabolic rate and protein solubility Figure 1 shows the plot of linearity between

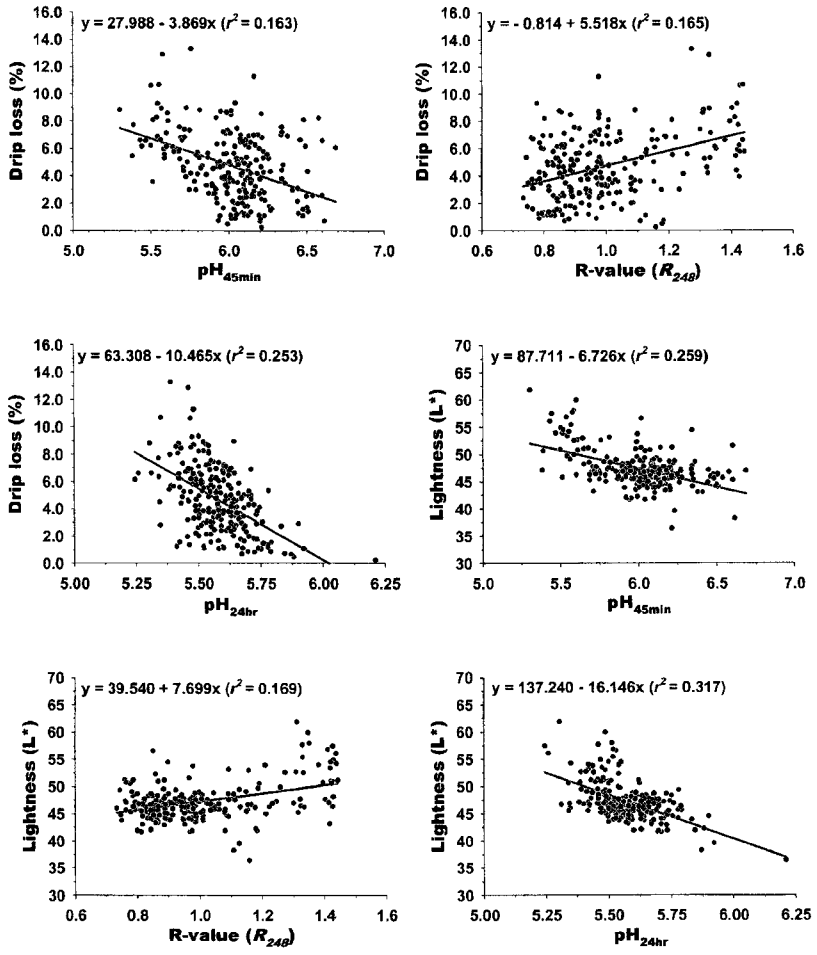

Fig. 1. Relationships of drip loss and lightness to pH_{45min}, R-value, and ultimate muscle pH in pig *longissimus dorsi* muscle. The regression lines were drawn from equations.

drip loss or lightness and the corresponding components. The muscle pH_{45min}, R-value, and pH_{24hr} were selected to estimate the regression equation model of drip loss and lightness, although the variances of coefficient estimates could account for only a small part of drip loss (about 16.3 to 25.3%) and lightness (about 16.9 to 31.7%). The muscle pH_{24hr} was represented to drip loss and lightness, which explained the corresponding estimations of 25.3 and 31.7 % in drip loss and lightness, respectively. Warriss and Brown (13) reported that drip loss is related to the ultimate muscle pH, although this only explained the corresponding 14.6% of the estimation in the exudates, and lower ultimate pH values are associated with higher reflectance values. However, only 14.8% of the estimation in color was explained by the pH. Therefore, the ultimate muscle pH alone accounts for only a small part of drip loss and lightness.

Table 1 shows the regression equations for the prediction of drip loss and lightness. The combination of the prediction equation using the muscle pH_{24hr} and protein solubility measured 24 hr PM explained the corresponding 43.5 and 59.8% of the estimation in drip loss and lightness, respectively. Lopez-Bote *et al.* (14) asserted that the solubility of sarcoplasmic protein may function as a better indicator for muscle quality, and may induce increased drip in the PSE pork. Therefore, decreases in the muscle pH and protein solubility significantly influence the meat quality, as well as drip loss and lightness.

The variances of coefficient estimates in drip loss and lightness explained by the early PM metabolic rate (pH_{45min} and R-value) were only 18.9 and 26.2%, respectively (Table 1). The early PM metabolic rate contributed significantly to the prediction of the pork quality (15, 16). However, the equations predicting drip loss and lightness from the corresponding components of the pH_{45min} and R-value were less useful than the ones based on the ultimate muscle pH and protein solubility measurements. The variances of coefficient estimates in drip loss and lightness explained by the early PM protein solubility measurements were 17.4 and 43.1%, respectively. The early PM protein denaturation contributed significantly to predicting the drip loss (2, 17); however, protein denaturation within 1 hr PM accounted for only a small part of drip loss at 24 hr PM. In contrast, protein denaturation during the early PM period had a large influence on the lightness. Similar tendencies were observed using a combination of the early PM metabolic rate and the protein solubility. Therefore, the protein solubility measured 45 min PM could be used for the prediction of lightness, and the muscle reflectance and exudation vary to some degree independently.

Histochemical characteristics Figure 2 shows the results of the relationships and the linear regression between the metabolic rate or meat quality traits and percentage area of the type IIb fiber. The area percentage of type IIb fiber is related to the metabolic rate and thereby the meat quality (6, 9). The area percentage of the type IIb fibers contributed significantly to the prediction of metabolic rate and meat quality; however, the variances of coefficient estimates accounted for only a small part of the metabolic rate and meat quality traits. Similar results were observed in the linear regression between the protein solubility measurements and the area percentage of the type IIb fiber (Fig. 3). The type I and IIb fibers, also known as slow-oxidative and fast-glycolytic, respectively, represent two extreme metabolic profiles, and the type IIa and IIx fibers are intermediate fibers (18). Because muscle fibers contain different myosin heavy chains, which are responsible for their different ATPase activity (19), it is possible that fiber type composition may be associated with the PM changes in the conversion of muscle to meat and subsequently the meat quality.

Table 2 shows the regression equations for the prediction of metabolic rate, protein solubility, and meat quality. A prediction equation using the combination of area percentage of type IIb fiber and number percentage of type I fiber explained the corresponding 13.3 and 12.6% of the estimation in drip loss and lightness, respectively. The variances of coefficient estimates in the protein solubility explained by the muscle fiber characteristics were only 10.2 to 19.5%. Stepwise regression equations using all possible combinations of the histochemical variables showed similar tendencies (Table 3). Therefore, the area percentage of the type IIb fiber alone accounts for

Table 1. Regression equations using metabolic rate and protein solubility to predict drip loss and lightness of pig *longissimus dorsi* muscle

Prediction equations	R^2	P value
Regression using pH_{24hr} and protein solubility measured at 24 hr PM		
Drip loss = 51.750 – 6.507·(pH_{24hr}) – 0.050·(TPS) – 0.020·(SPS)	0.451	< 0.0001
Lightness = 107.573 – 7.074·(pH_{24hr}) – 0.059·(TPS) – 0.138·(SPS)	0.596	< 0.0001
Regression using early postmortem metabolic rate		
Drip loss = 14.741 – 2.204·(pH_{45min}) + 3.226·(R_{248})	0.189	< 0.0001
Lightness = 81.515 – 5.947·(pH_{45min}) + 1.508·(R_{248})	0.262	< 0.0001
Regression using protein solubility measured at 45 min PM		
Drip loss = 15.879 – 0.023·(TPS) – 0.081·(SPS)	0.174	< 0.0001
Lightness = 71.545 – 0.060·(TPS) – 0.148·(SPS)	0.431	< 0.0001
Regression using pH_{45min}, R_{248}, and protein solubility measured at 45 min PM		
Drip loss = 16.251 – 1.487·(pH_{45min}) + 2.621·(R_{248}) + 0.002·(TPS) – 0.070·(SPS)	0.223	< 0.0001
Lightness = 94.455 – 3.672·(pH_{45min}) – 3.351·(R_{248}) – 0.059·(TPS) – 0.123·(SPS)	0.460	< 0.0001

Abbreviations used in the prediction equations: pH_{24hr}, muscle pH measured 24 hr PM; TPS, total protein solubility; SPS, sarcoplasmic protein solubility; pH_{45min}, muscle pH measured 45 min PM; R_{248}, R-value (A_{248}/A_{260}) measured 45 min PM.

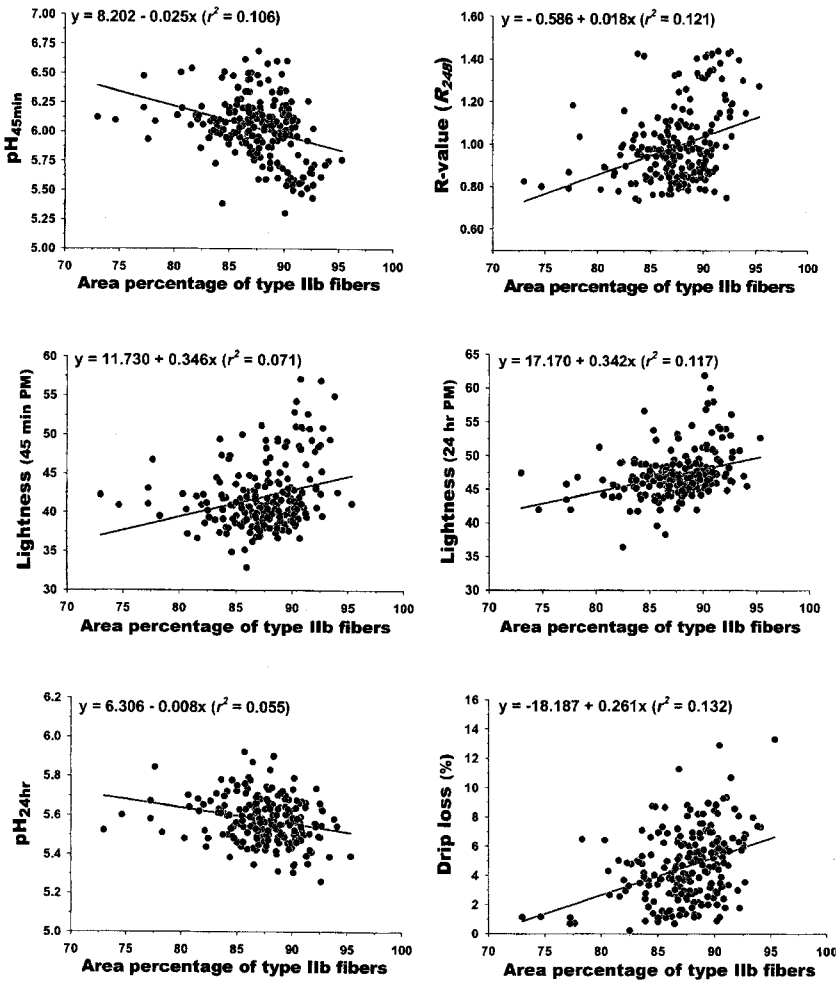

Fig. 2. Relationships of meat quality traits to area percentage of type IIb fiber in pig *longissimus dorsi* muscle. The regression lines were drawn from equations.

only a small part of the metabolic rate and protein denaturation, and the equations predicting the meat quality traits from the area percentage of type IIb fiber alone are less useful than the ones based on the early PM parameters. Hence, the muscle histochemical characteristics were not accurate enough, because the meat

Table 2. Regression equations using muscle fiber characteristics to predict metabolic rate, protein solubility, and meat quality traits of pig *longissimus dorsi* muscle

Prediction equations	R^2	*P* value
45 min postmortem		
pH_{45min} = 7.636 – 0.091·(APIIb) + 0.009·(NPI)	0.115	< 0.0001
R_{248} = –0.772 + 0.020·(APIIb) + 0.003·(NPI)	0.123	< 0.0001
Lightness = 20.628 + 0.257·(APIIb) – 0.143·(NPI)	0.078	0.0002
TPS = 362.126 – 1.773·(APIIb) + 0.330·(NPI)	0.115	< 0.0001
SPS = 135.348 – 0.680·(APIIb) + 0.393·(NPI)	0.175	< 0.0001
MPS = 226.762 – 1.092·(APIIb) – 0.063·(NPI)	0.057	0.0030
24 hr postmortem		
pH_{24hr} = 6.494 – 0.010·(APIIb) – 0.003·(NPI)	0.059	0.0016
Drip loss = –16.418 + 0.243·(APIIb) – 0.028·(NPI)	0.133	< 0.0001
Lightness = 24.604 + 0.268·(APIIb) – 0.119·(NPI)	0.126	< 0.0001
TPS = 439.781 – 2.832·(APIIb) – 0.485·(NPI)	0.172	< 0.0001
SPS = 168.692 – 1.114·(APIIb) – 0.232·(NPI)	0.195	< 0.0001
MPS = 271.072 – 1.718·(APIIb) – 0.253·(NPI)	0.102	0.0002

Abbreviations used in the prediction equations: APIIb, area percentage of type IIb fiber; NPI, number percentage of type I fiber.

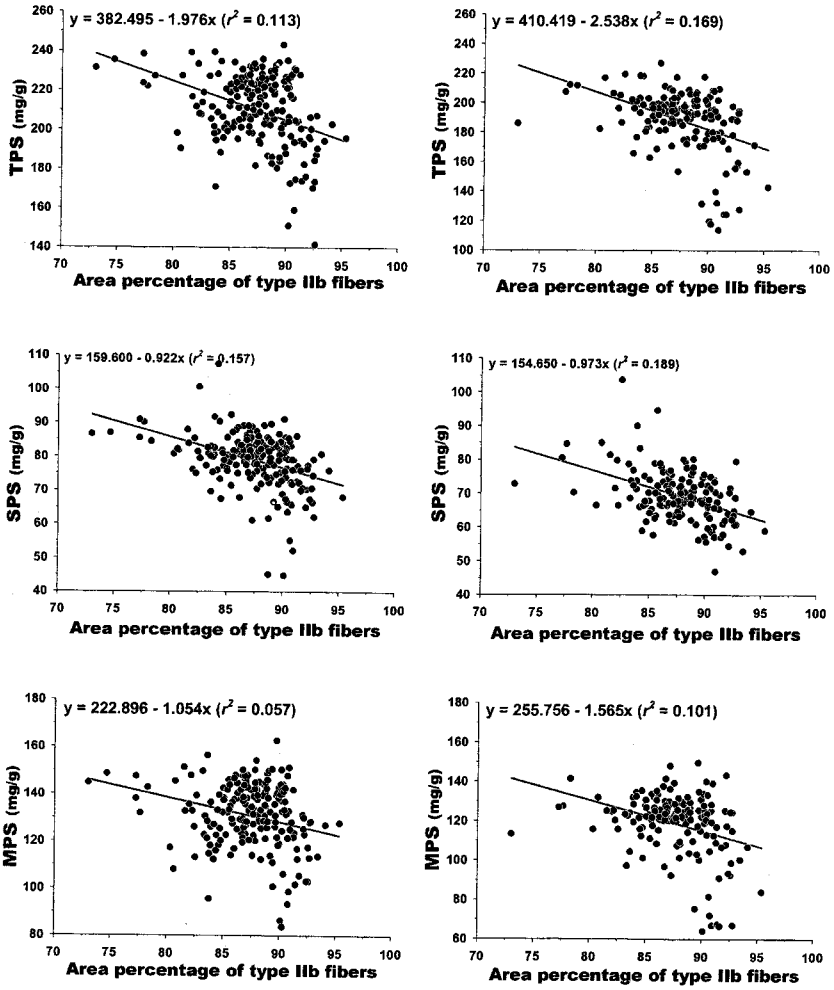

Fig. 3. Relationships of area percentage of type IIb fiber to protein solubility measured at 45 min PM (A) and 24 hr PM (B) in pig *longissimus dorsi* muscle. The regression lines were drawn from equations.

quality was not only affected by intrinsic factors such as the fiber type composition, but also by its interaction with the PM extrinsic factors. These results suggest that the estimated model using both the metabolic properties of

Table 3. Stepwise regression equations using fiber type composition to predict metabolic rate, protein solubility, and meat quality traits of pig *longissimus dorsi* muscle

Prediction equations	R^2	*P* value
45 min postmortem		
pH_{45min} = 22.304 – 0.143·(NPI) – 0.156·(NPIIa) – 0.166·(NPIIb)	0.120	< 0.0001
R_{248} = –11.169 – 0.027·(API) – 0.156·(NPIIa) + 0.129·(NPI) + 0.112·(NPIIa) + 0.124·(NPIIb)	0.140	< 0.0001
Lightness = –237.412 – 0.373·(API) + 2.737·(NPI) + 2.735·(NPIIa) + 2.834·(NPIIb)	0.088	0.0007
TPS = 1821.907 – 2.061·(APIIb) – 14.135·(NPI) – 14.664·(NPIIa) – 14.283·(NPIIb)	0.143	< 0.0001
SPS = 183.770 – 0.011·(API) – 1.145·(APIIb) – 0.385·(NPIIa)	0.183	< 0.0001
MPS = 1345.226 – 1.100·(APIIb) – 11.209·(NPI) – 11.256·(NPIIa) – 11.167·(NPIIb)	0.084	0.0017
24 hr postmortem		
pH_{24hr} = –14.550 + 0.203·(API) + 0.210·(APIIa) + 0.209·(APIIb) – 0.009·(NPIIb)	0.121	< 0.0001
Drip loss = –4351.341 + 43.363·(API) + 43.369·(APIIa) + 43.458·(APIIb) + 0.141·(NPIIb)	0.165	< 0.0001
Lightness = –229.807 – 0.130·(API) + 2.578·(NPI) + 2.649·(NPIIa) + 2.814·(NPIIb)	0.152	< 0.0001
TPS = 1917.111 – 2.177·(APIIb) – 15.342·(NPI) – 15.006·(NPIIa) – 15.438·(NPIIb)	0.208	< 0.0001
SPS = 130.884 + 0.999·(API) – 0.792·(NPI) – 0.757·(NPIIb)	0.201	< 0.0001
MPS = 1507.662 + 3.913·(API) – 15.501·(NPI) – 13.095·(NPIIa) – 14.117·(NPIIb)	0.172	< 0.0001

Abbreviations used in the prediction equations: API, area percentage of type I fiber; APIIa, area percentage of type IIa fiber; APIIb, area percentage of type IIb fiber; NPI, number percentage of type I fiber; NPIIa, number percentage of type IIa fiber; NPIIb, number percentage of type IIb fiber.

muscle and PM metabolic rate could be used to predict the pork quality traits.

Acknowledgments

This work was supported by Korea Science and Engineering Foundation grant program # R01-1999-000-00179-0.

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