Estimation of Correlation Coefficients between Histological Parameters and Carcass Traits of Pig *Longissimus Dorsi* Muscle

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ABSTRACT : The aim of this study was to investigate the histochemical parameters of muscle fibers, and to estimate the correlation between these histological parameters and carcass traits in pigs. A total of 230 crossbred Duroc×(Yorkshire×Landrace) pigs (149 gilts and 81 castrated male pigs) was evaluated. Carcass traits (carcass weight, backfat thickness, and loin eye area), muscle fiber size (cross-sectional area, diameter, and perimeter), muscle fiber number (density of fibers/mm^2 and total number of fibers), and fiber type composition (percentages of myofibers and relative areas of each fiber type) were evaluated. Mean cross-sectional area (CSA) and type IIB fiber CSA were positively correlated to carcass weight, backfat thickness and loin eye area. Mean fiber CSA was mostly related to type IIB CSA (r=0.98) as a result of the high percentage of type IIB fibers in the *longissimus* muscle. Correlations between fiber diameters and perimeters were also high, and showed similar results with CSA. Mean fiber density was negatively correlated to carcass weight (r=-0.24), backfat thickness (r=-0.18) and loin eye area (r=-0.27). To the contrary, total fiber number was positively correlated with carcass weight (r=0.27) and loin eye area (r=0.53). Carcass weight and loin eyZe area were not significantly related to muscle fiber composition. For backfat thickness, there was an opposition between type IIA percentage, which was positively related and type IIB percentage, which was negatively related. Fiber type composition of type I and IIA fibers were negatively correlated to that of type IIB fibers (r=-0.67 to -0.74). In the present study, carcass weight and loin eye area were positively correlated to CSA and negatively correlated to fiber density. But, these relationships were generally low. The fiber density was strongly affected by muscle fiber size and the total fiber number was affected either by CSA of muscle fiber and loin eye area. Fiber type composition was much more related to their numerical abundance than their CSA. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 428-433)***

Key Words : Carcass Traits, Skeletal Muscle, Muscle Fiber Characteristics

INTRODUCTION

One of the unique characteristics of skeletal muscle is its diversity in morphological and biochemical characters. In nature, muscles actually always comprise a mixture containing all of the fiber types (Chang et al., 2003). The predominant characteristic of a given muscle is determined by its relative composition in the different muscle fiber types. Muscle fibers are commonly categorized as types I (slow-twitch, oxidative), IIA (fast-twitch, oxido-glycolytic), or IIB (fast-twitch, glycolytic) (Brooke and Kaiser, 1970; Peter et al., 1972).

It is generally accepted that changes in fiber type composition affect the metabolic properties of a muscle, which influence muscle metabolism in the period around slaughter and thereby meat quality (Brocks et al., 2000). But, the relationship of fiber type composition to meat quality is not fully established for pigs (Karlsson et al., 1999).

Size and number of muscle fibers are also important parameters that affect muscle characteristics. Muscle fiber number is known to be an important determinant of muscle mass (Miller et al., 1975). Stickland and Handel (1986) suggested that the differences in muscle size are due to differences in muscle fiber number. Muscle growth potential in pigs is positively related to the total number of muscle fibers (Dwyer et al., 1993) and low muscle fiber number correlates with fibers which exhibit greater hypertrophy (Fiedler et al., 1993).

There are many parameters that express the muscle fiber characteristics. In general, muscle fiber composition is expressed as percentages of muscle fibers (Gentry et al., 2002) and relative areas (Larzul et al., 1997). Fiber size is evaluated by cross-sectional area (Brocks et al., 2000), diameter (Fiedler et al., 2003) or perimeter of each fibers. In order to evaluate the fiber number, it is usually used the density of fibers per mm^2 (Highley et al., 1999) or total number of fibers (Larzul et al., 1997; Fiedler et al., 2003).

It is well known that these histological and histochemical parameters are closely correlated to each other. Research on the relationships among these parameters is important for improving our understanding of the phenotypic expression in skeletal muscle. The aim of this study was to investigate the histochemical parameters of muscle fibers, and to estimate the correlation between these histological parameters and carcass traits in pigs.

MATERIALS AND METHODS

Animals

A total of 230 crossbred pigs (Duroc×[Yorkshire×

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Table 1. Means, standard deviations (SD) and ranges for all variables of the *longissimus dorsi* muscle

	Mean	SD	Min	Max
Carcass weight (kg)	82.73	7.97	62	103
Backfat thickness (mm)	20.64	5.97	8	38
Loin eye area (cm^2)	45.89	5.62	34.8	62.6
Cross-sectional area (μm^2)				
Mean	3,941	690	2,520	6,629
I	2,749	597	1,488	4,746
IIA	2,325	505	1,197	4,625
IIB	4,322	835	2,564	7,584
Diameter (μm)				
Mean	68.12	5.53	55.26	84.77
I	57.16	6.84	34.09	78.16
IIA	52.47	5.80	39.99	74.93
IIB	71.51	6.84	38.29	92.99
Perimeter (μm)				
Mean	238.7	20.4	191.6	312.1
I	193.5	21.5	140.7	257.8
IIA	183.1	20.0	141.9	258.7
IIB	251.9	23.9	193.5	339.1
Density of fibers per mm^2				
Mean	261.1	43.2	151	397
I	20.5	9.2	2	60
IIA	30.6	10.0	9	57
IIB	210.1	39.9	114	352
Total No. of fibers (×1,000)				
Mean	1,193.6	230.0	720	1,939
I	93.7	42.7	10	290
IIA	138.8	43.8	36	273
IIB	960.8	211.9	565	1,662
Percentages of myofibers				
I	7.89	3.34	1.38	20.26
IIA	11.88	3.97	3.34	24.89
IIB	80.24	4.85	65.43	92.12
Relative areas (%)				
I	5.48	2.25	0.62	14.74
IIA	6.98	2.45	1.91	19.86
IIB	87.55	3.31	73.01	95.35

Landrace], 149 gilts and 81 castrated male pigs) was evaluated. The abattoir used a traditional scalding-singeing process. After electrical-stunning, the carcasses were exsanguinated for 5 min before scalding in 65°C hot water. After evisceration carcass weight was measured and backfat thickness was also measured at the 11th and last *thoracic vertebrae*. The mean of these two measurements was used as backfat thickness.

Histological and histochemical analyses

Within 1 h after slaughter, muscle samples for histochemical analysis were taken from *longissimus* muscle at the 8th *thoracic vertebrae*. Samples were cut into pieces of 0.5×0.5×1.0 cm, promptly frozen in isopentane cooled by liquid nitrogen and stored at -80°C until subsequent analyses. Serial transverse muscle sections (10 μm) were obtained from each sample with a cryostat at -20°C and mounted on glass slides.

The sections were stained for myosin ATPase reactivity according to the methods of Brooke and Kaiser (1970). 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, adjusted to pH 4.7 with acetic acid (Lind and Kernell, 1991). After pre-incubation, the sections were subjected to the following steps: (1) washing in four rinses of distilled water; (2) washing for 30 s in a 20 mM glycine buffer (pH 9.4) containing 20 mM $CaCl_2$; (3) 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); (4) washing in three 30 s changes of 1% $CaCl_2$; (5) washing in 2% cobalt chloride for 3 min; (6) washing in three changes of distilled water; (7) immersing in 1% yellow ammonium sulfide for 30 s; (8) washing in several changes of distilled water; (9) mounting in glycerol jelly.

All histochemical samples were examined by an image

Table 2. Correlation coefficients (r) between carcass traits and muscle fiber size in the *longissimus dorsi* muscle

	Cross-sectional area				Diameter				Perimeter			
	Mean	I	IIA	IIB	Mean	I	IIA	IIB	Mean	I	IIA	IIB
Carcass weight	0.26	0.13	0.31	0.25	0.22	0.08	0.28	0.20	0.25	0.12	0.30	0.24
Backfat thickness	0.18	0.04	0.07	0.21	0.18	0.04	0.06	0.22	0.16	0.02	0.06	0.20
Loin eye area	0.25	0.20	0.28	0.25	0.22	0.11	0.23	0.10	0.24	0.19	0.27	0.17
Cross-sectional area												
Mean		0.49	0.67	0.98	0.92	0.41	0.59	0.89	0.97	0.48	0.63	0.96
I			0.55	0.39	0.55	0.97	0.58	0.42	0.51	0.97	0.53	0.39
IIA				0.59	0.65	0.50	0.95	0.54	0.70	0.56	0.97	0.61
IIB					0.88	0.32	0.51	0.91	0.94	0.38	0.55	0.98
Diameter												
Mean						0.58	0.69	0.97	0.94	0.56	0.65	0.91
I							0.58	0.41	0.45	0.98	0.48	0.31
IIA								0.56	0.66	0.60	0.96	0.55
IIB									0.90	0.41	0.52	0.93
Perimeter												
Mean										0.53	0.70	0.98
I											0.55	0.40
IIA												0.59

$r \geq 0.17$, significant at $p<0.05$.

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 controls all the image analysis system (Image-Pro Plus, Media Cybernetics, L.P., USA).

All portions of the sections analyzed were free from tissue disruption and freeze damage. About 600 fibers were evaluated. Muscle fibers were identified as type I, IIA and IIB by dark, light and intermediate staining, respectively. Percentage of myofibers is the ratio of counted fiber number of each fiber type to the total counted fiber number and relative area is the ratio of the total cross-sectional area (CSA) of each fiber type to the total measured fiber area. The average CSA, diameter and perimeter of type-identified fibers were also measured.

The density of fibers was calculated from the mean number of fibers per mm^2. From the fiber density and the CSA measures, the total number of fibers was calculated as the loin eye area×fiber density.

Statistical analysis

Descriptive statistics were performed using SAS PC software (SAS, 1996) to calculate means and standard deviations for all variables. Results are expressed as means, standard deviations and overall ranges. Overall correlation coefficients between the histological characteristics and carcass traits were evaluated using partial correlation coefficients (CORR procedure of SAS, 1996).

RESULTS AND DISCUSSION

Descriptive statistics

The means, standard deviations and overall ranges for all variables measured are given in Table 1. Those included carcass traits (carcass weight, backfat thickness and loin eye area), muscle fiber size (cross-sectional area, diameter and perimeter), muscle fiber number (density of fibers per mm^2 and total number of fibers) and fiber type composition (percentages of myofibers and relative areas of each fiber type).

To express the fiber type composition, two calculation methods were compared. The numerical percentages occupied by type I, IIA or IIB fibers were 7.89±3.34%, 11.88±3.97% and 80.24±4.85%, respectively. While, the relative areas of each fiber type were 5.48±2.25%, 6.98±2.45% and 87.55±3.31%, respectively.

The Percentage of type IIB fibers ranged from 65.43 to 92.12% and the relative area of type IIB fibers ranged from 73.01 to 95.35%. Because the cross-sectional area (CSA) of type IIB fibers (4,322 μm^2) was greater than that for the type I (2,749 μm^2) and IIA (2,325 μm^2) fibers, the relative area of type IIB fibers was higher than that calculated with fiber number percentage method.

According to fiber type composition, as would be expected, the *longissimus dorsi* muscle was fast-twitch muscle. There are marked differences in fiber type composition, fiber areas and metabolic profiles among muscles both within and between pigs (Klont et al., 1998). M. *longissimus dorsi* (LD) has a large proportion of type IIB fibers (80-90%), while *quadriceps femoris* contains a higher percentage of type I fibers and has a higher oxidative capacity than *longissimus* muscle (Rosser et al., 1992).

The histochemical profile of LD muscle of the Duroc×(Yorkshire×Landrace) pigs used in the present experiment is close to that of the Yorkshire pigs (6.4, 5.3, 88.3%) and that of the Landrace pigs (6.9, 4.9, 88.2%; Ruusunen and Puolanne, 1997).

Table 3. Correlation coefficients (r) between carcass traits, muscle fiber size and muscle fiber number in the *longissimus dorsi* muscle

	Density of fibers per mm^2				Total number of fibers			
	Mean	I	IIA	IIB	Mean	I	IIA	IIB
Carcass weight	-0.24	–.02	-0.12	-0.23	0.27	0.18	0.19	0.22
Backfat thickness	-0.18	-0.11	0.11	-0.21	-0.12	-0.16	0.26	-0.16
Loin eye area	-0.27	-0.07	-0.25	-0.26	0.53	0.21	0.17	0.52
Cross-sectional area								
Mean	-0.97	-0.36	-0.22	-0.93	-0.76	-0.31	-0.18	-0.76
I	-0.51	-0.37	-0.21	-0.43	-0.37	-0.34	-0.17	-0.34
IIA	-0.64	-0.05	-0.31	-0.62	-0.44	-0.02	-0.20	-0.43
IIB	-0.95	-0.31	-0.17	-0.94	-0.74	-0.26	-0.05	-0.77
Density of fibers/mm^2								
Mean		0.35	0.29	0.96	0.78	0.30	0.20	0.78
I			-0.03	0.12	0.33	0.97	-0.05	0.06
IIA				0.06	0.07	-0.11	0.92	-0.10
IIB					0.76	0.13	0.01	0.82
Total No. of fibers								
Mean						0.41	0.30	0.97
I							0.01	0.24
IIA								0.13

$r \geq 0.17$, significant at $p<0.05$.

Correlation between carcass traits and muscle fiber size

The correlation coefficients between carcass traits and muscle fiber size are presented in Table 2. Mean CSA and type IIB fiber CSA were positively correlated to carcass weight, backfat thickness and loin eye area. Among fiber types, type IIA fiber showed higher relationship with carcass weight (r=0.31) and loin eye area (r=0.28) but was not significantly correlated with backfat thickness. Muscle fiber diameter and perimeter were the same relationship as CSA. The overall relationship between carcass traits and muscle fiber size was generally low.

As expected, all correlations between each fiber type CSA were significantly positive and ranged from 0.39 to 0.98, in agreement with Larzul et al. (1997). Mean fiber CSA was mostly related to type IIB CSA (r=0.98) as a result of the high percentage of type IIB fibers in the *longissimus* muscle. CSAs of both type I and IIA fibers were positively correlated to that of type IIB fibers (r=0.39, 0.59, respectively). Candek-Potokar et al. (1999) suggested that increase in size of one fiber type was accompanied by enlargement of all fibers, regardless of their type.

Correlations between fiber diameters and perimeters were also high, and showed similar results with CSA. The high positive correlation between the sizes of different fiber types agrees with other results in pig (Larzul et al., 1997; Candek-Potokar et al., 1999).

Muscle fiber number

The overall relationship between carcass traits and muscle fiber density was generally low (Table 3). Mean fiber density was negatively correlated to carcass weight (r=-0.24), backfat thickness (r=-0.18) and loin eye area (r=-0.27). Among fiber types, type IIB fiber number showed higher relationship with carcass weight (r=-0.23), backfat thickness (r=-0.21) and loin eye area (r=-0.26). The negative correlation observed between mean fiber density and carcass weight can be explained by the positive relationship between mean CSA and carcass weight (Table 2), and by the negative relationship between CSA and fiber density. To the contrary, total fiber number was positively correlated with carcass weight (r=0.27) and loin eye area (r=0.53). The total number of fibers was calculated as the loin eye area to multiply fiber density and these two parameters were affected by a simultaneous effect of carcass weight. Loin eye area was also affected by total number and length of muscle fibers (Candek-Potokar et al., 1999).

The density of fibers was calculated from the mean number of fibers per mm^2. So, fiber density was negatively related to mean CSA (r=-0.97), with values ranging from -0.51 to -0.95 depending on the fiber type (Table 3). Total fiber number was also negatively related to mean CSA (r=-0.76), with values ranging from -0.37 to -0.74 depending on the fiber type. These results indicated that the fiber density was strongly affected by muscle fiber size and the total fiber number was affected either by CSA of muscle fiber and loin eye area.

Mean fiber density was mostly related to type IIB fiber number (r=0.96) as a result of the high percentage of type IIB fibers and showed low relationship with type I and IIA fibers (r=0.35, 0.29, respectively). Henckel et al. (1997) suggested that a higher muscle gain may be explained either by a higher level of hypertrophy of the individual fibers and/or a higher total number of fibers. The results of the present study indicate that carcass weight and loin eye area were related to a high CSA and the loin eye area was also closely related to total number of fibers.

Table 4. Correlation coefficients (r) between muscle fiber size, number and fiber type composition in the *longissimus dorsi* muscle

	Percentages of myofibers			Relative areas		
	I	IIA	IIB	I	IIA	IIB
Carcass weight	0.07	0.03	-0.08	0.05	0.08	-0.09
Backfat thickness	-0.13	0.34	-0.20	-0.16	0.26	-0.07
Loin eye area	0.12	-0.13	0.10	0.05	-0.09	0.05
Cross-sectional area						
Mean	0.03	0.36	-0.27	-0.09	0.21	-0.08
I	-0.22	0.05	0.06	0.17	0.12	-0.20
IIA	0.17	0.09	-0.21	0.21	0.35	-0.40
IIB	0.09	0.45	-0.40	-0.08	0.26	-0.13
Density of fibers/mm^2						
Mean	-0.05	-0.33	0.26	0.07	-0.19	0.08
I	0.93	-0.35	-0.46	0.90	-0.08	-0.53
IIA	-0.25	0.85	-0.59	-0.15	0.78	-0.48
IIB	-0.23	-0.49	0.54	-0.08	-0.37	0.33
Total No. of fibers						
Mean	-0.05	-0.34	0.30	0.07	-0.22	0.11
I	0.93	-0.34	-0.42	0.90	-0.10	-0.53
IIA	-0.13	0.84	-0.56	-0.15	0.80	-0.48
IIB	-0.20	-0.50	0.53	-0.07	-0.37	0.32
Percentages of myofibers						
I		-0.01	-0.67	0.90	-0.19	-0.57
IIA			-0.74	-0.02	0.88	-0.71
IIB				-0.58	-0.67	0.83
Relative areas						
I					-0.13	-0.69
IIA						-0.73

r>0.17, significant at p<0.05.

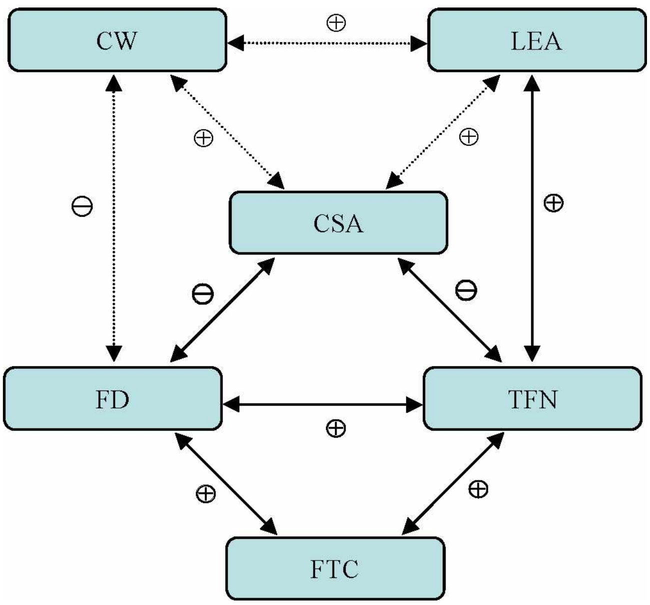

Figure 1. Schematic representation of the relationship between carcass traits and histological characteristics in pigs. CW, carcass weight, LEA, loin eye area, CSA, cross-sectional area of muscle fiber; FD, fiber density per mm^2; TFN, total fiber number; FTC, fiber type composition. Bold represent indicated moderate to high correlation.

Muscle fiber composition

Fiber type composition of type I and IIA fibers were negatively correlated to that of type IIB fibers (r=-0.67, -0.74, respectively; Table 4). The absence of correlation between type I and IIA fibers suggests that less type I fibers is not compensated by more type IIA fibers in accordance with Candek-Potokar et al. (1999).

Relative area of a given fiber type was highly correlated to the corresponding fiber type percentage (r=0.83 to 0.90), and poorly related to the corresponding CSA (r=-0.13 to 0.35). Each fiber type density was highly correlated to the corresponding fiber type percentage (r=0.54 to 0.93) and relative area (r=0.33 to 0.90). In the present study, fiber type composition was much more related to their numerical abundance than their CSA, in accordance with Larzul et al. (1997).

Low correlations were found between carcass traits and muscle fiber composition (r=-0.20 to 0.34). Carcass weight and loin eye area were not significantly related to muscle fiber composition. For backfat thickness, there was an opposition between type IIA percentage, which was positively related (r=0.34) and type IIB percentage, which was negatively related (r=-0.20).

Overall relationships between carcass traits and muscle fiber characteristics are presented in Figure 1. Carcass weight was positively correlated to loin eye area and CSA, and negatively correlated to fiber density. But, these relationships were generally low. Loin eye area showed similar results with carcass weight, but it was more closely related to total fiber number. So, a larger loin eye area may be explained by a higher total number of fibers.

IMPLICATIONS

Carcass weight and loin eye area were positively correlated to CSA, and negatively correlated to fiber density. But, these relationships were generally low. The fiber density was strongly affected by muscle fiber size and the total fiber number was affected either by CSA of muscle fiber and loin eye area. Fiber type composition was much more related to their numerical abundance than their CSA.

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