Characterization of muscle fiber type in the pectoralis major muscle of slow-growing local and commercial chicken strains

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ABSTRACT The study aimed to characterize muscle fiber type of the pectoralis major muscle of slow-growing chickens belonging to the Padovana local breed, the commercial strain Berlanda gaina, and their cross. Forty-five chickens (both males and females) from the different genotypes were grown up to 180 d. Histochemical and morphometrical analyses were performed to characterize muscle fiber types, myofiber density, and myofiber size of the different genotypes. The effects of genotype, sex, and their interaction were estimated. Muscle samples appeared almost entirely made up of IIB fiber type, whereas a low percentage of area (5 to 6%) was composed of hypercontracted fiber. Myofiber density was significantly higher ($P < 0.05$) in Padovana strains and cross-sectional area was significantly lower ($P < 0.01$) than in Berlanda strain. Muscle fiber characteristics appeared not to be affected by the interaction of genotype × sex.

Key words: Padovana chicken, Berlanda strain, muscle fiber characteristic, genotype, sex

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INTRODUCTION

The need to produce a high quantity of meat to support increasing human demand over the world oriented genetic selection toward the production of commercial chickens that are the broiler, chicken meat type, characterized by a high and fast growth rate and by increased breast muscle resulting from an increase in fiber number and diameter (Iwamoto et al., 1993; Remignon et al., 1994, 1995; Burke and Henry, 1997). Concerning meat quality, negative effects have been reported, including the appearance of many "giant" hypercontracted fibers (**HF**). Giant fibers (**GF**) were for the first time reported by Cassens et al. (1969) in porcine muscles. Giant fibers are not considered a particular fiber type, but consist of fibers exhibiting structural and metabolic anomalies as an hypercontraction state that continues after resolution of rigor mortis (Sink et al., 1986; Dransfield and Sosnicki, 1999). Giant fibers have never been observed either in a biopsy sample (Solomon and Eastridge, 1987) or in prerigor muscle (Remignon et al., 2000). Giant fibers have been reported in a cross-section of muscle sample collected postmortem in chickens (Klosowska et al., 1979), turkeys (Sosnicki et al., 1989), pigs (Handel and Stickland, 1986), cattle (Sink et al., 1986), and rabbits (Dalle Zotte et al., 2001). Giant fibers presence is generally associated with selection for meat production (Dransfield and Sosnicki, 1999). In turkey a higher GF percentage was reported in fast-growing hybrids (Remignon et al., 2000). In chickens, fast-growing lines are reported to have pectoralis muscle fibers larger than slow-growing chickens; larger fiber size is often associated with an increased number of GF (Dransfield and Sosnicki, 1999; Le Bihan-Duval, 2003; Miraglia et al., 2006). Numerous studies underlined a negative relation between GF and meat quality in pigs (Fiedler et al., 1999; Fazarinc et al., 2002) and in turkeys (Barbut, 1993, 1997; Sosnicki et al., 1998), whereas a clear relation between GF and meat quality was not established in chickens.

In the last years, the biodiversity safeguards (Council Regulation EC 834/2007; http://ec.europa.eu/agriculture/organic/eu-policy/legislation_en) encouraged the development of organic rearing of poultry. Moreover, consumer requests for quality meat with low environmental impact (Sundrum, 2001) encouraged use of local breeds as a traditional product for the local market. The Padovana chicken is a local fancy breed primarily used for ornamental purpose because of a big forelock decorating its head. The Padovana breed is a slow-growing chicken that reaches sexual maturity at approximately 180 d of age in a low-input production system (De Marchi et al., 2005). The characterization of Padovana chicken breeds was largely studied using genomic and proteomic studies in the recent years (De Marchi et al., 2006; Soattin et al., 2009; Zanetti et al., 2010a, 2011a,b). The Padovana chicken provides lean

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carcasses with moderate meat yield and is particularly appreciated for flavor, succulence, and tenderness of its meat (Zanetti et al., 2010b; De Marchi et al., 2011). From a nutritional point of view, meat appears characterized by low lipid content and high content of polyunsaturated fatty acids (De Marchi et al., 2005, 2012; Riovanto et al., 2012).

The aim of this study was to characterize muscle fiber in the pectoralis major muscle of the Padovana local chicken breed, a commercial slow-growing strain, and their cross.

MATERIALS AND METHODS

This project was approved by the Ethical Committee for the Care and Use of Experimental Animals (Comitato Etico di Ateneo per la Sperimentazione Animale; http://www.unipd.it/comitato-etico-di-ateneo-la-sperimentazione-animale-ceasa) of the University of Padova (Legnaro, Italy).

Birds, Slaughter Procedures, and Sample Collection

Forty-five chickens, males (n = 20) and females (n = 25), from 3 different genotypes, 2 Padovana local breed varieties [Padovana argentata (**Pa**) and Padovana camosciata (**Pc**)], a commercial slow-growing line Berlanda gaina (**B**), and the cross **Pc×B**, were reared at the experimental farm of the Department of Agronomy, Food, Natural Resources, Animals and Environment (Legnaro, Italy). The animals number analyzed was n = 5 males and n = 5 females for each of the 5 genotypes sampled, except for Pa, which considered only the females.

One-day-old Padovana chicks were obtained from the Padovana nucleus flock of the Agricultural High School "Duca degli Abruzzi" in Padova. Berlanda chicks were obtained from "Avicola Berlanda" (Carmignano di Brenta, Padova, Italy). All the birds were housed in indoor pens, with natural light in conditions of decreasing photoperiod. Feed and water were supplied ad libitum. Two females died during the trial (one Pc×B and one Pc×Pa). At maturity (180 d), a total of 43 chickens, 20 males and 23 females, were weighed and brought to the slaughterhouse. Feed was withdrawn 18 h before slaughter. The birds were electronically stunned in a water bath (240 mA, 120 V, 5 s), killed by neck cut, exsanguinated, plucked, and eviscerated. After evisceration, whole carcasses were air chilled (airflow of 7 m^3/s, 0°C) and stored at 2°C. The length of the time between hanging birds and chilling carcasses was of about 15 min.

About 2 h after slaughter, samples of muscle pectoralis major were collected from the left breast, in the anterior area, in the third superior, at about 2 cm from median line. A similar location was sampled for all 5 genotypes. Samples were frozen in isopentane cooled by liquid nitrogen (Dubowitz and Brooke, 1973) and stored at −80° until histochemical analysis.

The analytical determination of shear force and cooking loss percentage were performed 24 h postmortem as previously reported by Zanetti et al. (2010b).

Histochemical Analysis and Image Analysis

Pieces of frozen muscle samples were mounted on cryostat chuck, equilibrated at −25°C, and cut. Serial sections were stained using hematoxylin/eosin staining, myosin ATPase method (Brooke and Kaiser, 1970) after acid preincubation (pH 4.30, 4.35, 4.40) and alkaline preincubation (10.40, 10.45). Determination of succinate dehydrogenase activity (**SDH**) was also performed (Pearse, 1972).

Images were acquired and analyzed with a semiautomatic image analysis system (Axiovision Digital Image Processing Software, Carl Zeiss, www.zeiss.de/axiovision). The following parameters were computed: density (fibers number/field), cross-sectional area (**CSA**) of each fiber type (μm^2), and percentage of field occupied by the different fiber types. An average of 150 muscle fibers/sample from 2 slide fields representative of the whole sample was analyzed. Every field measured about 240×10^3 μm^2.

Statistical Analyses

The data were analyzed statistically with the GLM procedure of SAS package (SAS Institute Inc., 1990) using a linear type III model considering genotype (**G**), sex (**S**), and the genotype × sex interaction (**G×S**). Due to the nonsignificant effect of the interaction G×S, the least squares means reported in the results were based on the linear model that considered only the 2 main effects of sex and genotype. The slide field effect was considered in the model as repeated records. Orthogonal contrasts were studied for genotypes B vs. (Pa×Pc) and for genotypes Pc vs. (Pc×B).

RESULTS AND DISCUSSION

Carcass characteristics and some qualitative meat traits are summarized in Table 1. Maximum shear force values, as measured on cooked breast muscle, were similar in the different genotypes, whereas cooking loss values were significantly lower ($P < 0.0001$) in the B strain than in the Pa breed. Sex affected both shear force ($P < 0.05$) and cooking loss ($P < 0.01$), which was lower in the male than in the female.

Results concerning analyses of variance on muscle fiber types, fiber density, and fiber size (CSA) of pectoralis major muscle in the different strains are reported in Table 2.

Pectoralis major muscle in Padovana breed and Berlanda hybrid is almost entirely made up of IIB fiber type (fast-twitch glycolytic) as reported in chicken breast muscle (Smith and Fletcher, 1988; Iwamoto et

Table 1. Least squares means of sex and genotype effects on meat traits in different chicken genotypes

	Sex		Genotype					P-value of contrasts		
Meat trait	Male 1	Female 2	Berlanda (B)	Padovana Argentata (Pa)	Padovana Camosciata (Pc)	Cross (Pc×B)	Cross (Pc×Pa)	B vs. Pa+Pc	Pc vs. Pc×B	RMSE[1]
Live weight, g	2,539[A]	1,804[B]	2,953	1,696	1,900	2,435	1,872	<0.0001	<0.0001	259
Dressing, %	79.2[A]	74.0[B]	75.7	76.2	76.9	78.0	76.0	0.2400	0.2349	4.71
Breast weight, g	358.3[A]	267.4[B]	430.5	230.9	270.7	371.3	260.8	<0.0001	<0.0001	53
Breast, %	17.8[a]	19.9[b]	19.4	18.1	18.3	19.8	18.4	0.0009	0.0004	2.24
Shear force, N	14.9[a]	15.7[b]	15.5	15.4	15.4	15.5	14.9	0.8192	0.8237	3.37
Cooking loss, %	18.6[A]	21.4[B]	18.1	21.8	20.3	18.7	21.2	<0.0001	0.0017	2.78

[a,b]Within a row, means not sharing a common superscript are significantly different at $P < 0.05$.

[A,B]Within a row, means not sharing a common superscript are significantly different at $P < 0.01$.

[1]RMSE = root mean square error.

al., 1993; Roy et al., 2006; Petracci and Cavani, 2012). Some fibers exhibited histological and histochemical features of GF. These fibers exhibited an oval or round shape and were often found grouped or isolated and generally located at the periphery of a fascicle. These fibers stained darker than other fibers when colored with hematoxylin-eosin, and moreover exhibited high ATPase activity, which had succinic dehydrogenase activity as high as previously reported (Solomon and West, 1985; Handel and Stickland, 1986). The CSA of these fibers was not so large to call them GF, so we called them HF.

The percentage of area occupied by HF in the pectoralis major muscle resulted between 6.16% in B, 5.58% in Pa, and 5.13% in Pc; the difference was not statistically significant so the distribution of HF in area (%) was not affected by genotype. Sex did not affect the distribution of HF in area. Hypercontracted fibers were on average larger than IIB fibers, whereas their size appeared similar in the different strains as in the 2 sexes.

Concerning myofiber density, in the same area fibers were more numerous ($P < 0.05$) in the Padovana breed, Pc (79.90) and Pa (85.59), and in the relative cross Pc×Pa (82.44) than in the B strain (65.07). Finally, the fiber CSA was significantly lower ($P < 0.01$) in Pc (2,444.97 μm^2) and Pa (2,237.56 μm^2) than in the B strain (2,934.75 μm^2). Sex did not affect myofiber density, whereas it affected fiber size, which was higher in the female than in the male ($P < 0.05$).

In this trial, based on a conventional rearing system, the pectoralis major muscle was totally made up of the IIB fiber type, both in Padovana chicken and the medium-growing chicken Berlanda strain.

Results showed that neither genotype nor sex affected the number of HF in the pectoralis major muscle. The HF percentage appeared equally exhibited in the different strains. The presence of HF is generally associated with selection for meat production (Solomon et al., 1998; Remignon et al., 2000). In the chicken, Miraglia et al. (2006) found that both Ross and Kabir hybrid have HF; nevertheless, the percentage of HF in the pectoralis major muscle was higher ($P < 0.001$) in the Ross. The presence of HF both in the Padovana chicken and Berlanda strain evidently is not associated with meat production, but it might be due to an intrinsic susceptibility of birds to metabolic stress occurring at slaughter (Holm and Fletcher, 1997; Debut et al., 2003; Ali et al., 2008; Chabault et al., 2012). Debut et al. (2003) reported that struggle activity on the shack line was higher in slow-growing lines, resulting in an accelerated rate of postmortem glycolysis that is detrimental for breast meat quality.

Table 2. Least squares means of sex and genotype effect on muscle fiber traits in chicken breast samples[1]

	Sex		Genotype					P-value of contrasts		
Item	Male 1	Female 2	Berlanda (B)	Padovana Argentata (Pa)	Padovana Camosciata (Pc)	Cross (Pc×B)	Cross (Pc×Pa)	B vs. Pa+Pc	Pc vs. Pc×B	RMSE
IIB density	78.82	71.38	65.07	85.59	79.90	62.50	82.44	0.0141	0.0350	16.36
HF density	2.78	2.16	2.71	3.06	2.81	1.67	2.10	0.7529	0.1701	1.68
IIB CSA, μm^2	2,443[a]	2,857[b]	2,935	2,238	2,445	3,209	2,422	0.0080	0.0034	505
HF CSA, μm^2	3,604	4,725	4,701	3,835	3,685	4,978	3,624	0.2564	0.1794	1,940
% area IIB	94.91	97.25	93.84	94.42	94.87	95.78	96.51	0.6082	0.6162	3.68
% area HF	5.09	4.75	6.16	5.58	5.13	4.22	3.49	0.6082	0.6162	3.68

[a,b]Within a row, means not sharing a common superscript are significantly different at $P < 0.05$.

[1]Density = fiber number/field; CSA = cross-sectional area (μm^2); % area = distribution of fiber type in area; HF = hypercontracted fiber; IIB = fiber type. RMSE = root mean square error.

Negative effects of HF concerning meat quality have been reported in the turkey (Barbut, 1993, 1997; Sosnicki et al., 1998). Grey et al. (1986) reported that in the turkey hypercontraction of muscle fibers is associated with toughening of the muscles. In the chicken, a clear relation between GF and meat quality was not established.

Cross-sectional area of IIB fiber type was significantly affected by genotype (Table 2). Cross-sectional area was lower in the Padovana chicken than in the Berlanda strain. These findings are in agreement with Burke and Henry (1997), Lonergan et al. (2003), and Chen et al. (2007) who reported small fiber size in slow-growing chicken strains. The increase in myofiber CSA is often associated with selection for meat production (Remignon et al., 1995; Dransfield and Sosnicki, 1999; Chen et al., 2007). In turkeys, the increase of fiber size had detrimental effects on meat quality (Berri, 2000). In chicken, tenderness is reported to be higher in breeds characterized by small CSA (Lonergan et al., 2003; Chen et al., 2007; Branciari et al., 2009; Zhao et al., 2011). Berri et al. (2007), in a study carried out in broiler breast muscle, even showed a positive influence of myofiber CSA on meat quality traits, as water-holding capacity and tenderness. Otherwise, Duclos et al. (2007) reported that pectoralis major muscle exhibiting larger fiber CSA was potentially better adapted to further processing than muscle exhibiting smaller fiber CSA. Finally, Choi and Kim (2009) stated that birds characterized by a greater number of moderate-size fibers provide a high amount of good quality meat. Our data pointed out a negative relation between fiber size and cooking loss as reported by Berri et al. (2007), whereas fiber size seemed not to affect tenderness.

The contrasting data reported in different studies might be partially due to the different breed analyzed but also to the stunning method used. Stressors before slaughter affect postmortem metabolism of the muscle tissue, which influences the characteristic of the meat.

In conclusion, results of this study provided a first characterization of the muscle fiber characteristics of both the Padovana breed and Berlanda Gaina line.

The pectoralis major muscle was totally made up of the IIB fiber type, both in the Padovana chicken and the medium-growing chicken Berlanda strain. Moreover, all of the strains of this study, Padovana, Berlanda, and their crosses, were characterized by the presence of HF, probably due to preslaughter stressful conditions affecting postmortem metabolism. The difference between the Padovana chicken and Berlanda strain was a higher myofiber density in the Padovana chicken and smaller myofiber size in the Padovana breed. Small fiber size is associated with a thin-grained meat that positively affects the sensorial perception of taste. The small fiber size of Padovana myofibers might partially support the good taste of Padovana meat. Data on the extracellular matrix, especially connective tissue, are not available for these breed. More information is needed to evaluate the traits affecting meat quality. Future studies are required to ascertain the role of other muscle components as intramuscular connective tissue and adipocytes.

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