GIANT FIBRES IN BROILER CHICKENS

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Background

Giant fibres can be described as being generally rounded in shape, bigger than the normal fibres around them, the shape of which can also be modified by them, and usually slightly separated from the surrounding fibres and localized at the borders of muscle fascicles. This is a great simplification of the problem as proven by the fact that their real origin and meaning has been the subject of discussion for over 30 years and still there is no clear answer to the question.

The issue of giant fibres was first dealt with by Cassens et al. in 1969. These authors focused their attention mainly on the frequency and on the histochemical characteristics of such fibres in the muscles of adult and growing pigs. Considering their particular staining pattern following histochemical evaluation as well as the fact that they were more frequent in muscles taken from stress-sensible pigs, these authors reached the conclusion that giant fibres may represent a pathological muscle modification. In later years other authors (Handel et al., 1986) focused their attention on giant fibres also observing them with the electron microscope which revealed, among other things, a reduced quantity of sarcoplasmic reticulum and an irregular disposition of the miofilaments inside the myofibres. This last point, as well as the lack of the characteristic myofibrillar banding pattern seen on examination of longitudinal sections, might be explained by supercontraction. These authors concluded that giant fibres might be due to a defect in single developing muscle fibres and not to degenerative changes within the muscle. A recent study by Fazarinc et al. (2002) deals with the influence of the halothane gene on the percentage of giant fibres. These authors reached the conclusion that giant fibres are glycolitic fibres which, due to the accumulation of intracellular lactate during post-mortem glycolisis, become swollen. Their appearance may also be facilitated by stress or stress susceptibility.

All of the above articles deal with pigs, but this is by no means the only animal where giant fibres can be found, as a matter of fact they have been observed in muscles of various species such as cattle and poultry. In this regard there is a paper (Remignon et al., 2000) concerning the moment, post-mortem, in which giant fibres appear in turkey muscles. These authors only found giant fibres in post-rigor (24h post-mortem) and not in pre-rigor (3 minutes post-mortem) muscles and therefore hypothesized that they are due to alterations in the developing muscle fibres that lead to structural and metabolic anomalies that give origin to their appearance during the biochemical events typical of rigor mortis.

Objectives

The purpose of this study is to evaluate the number as well as the histological and histochemical characteristics of giant fibres in various chicken muscles since literature concerning this topic is quite scarse. Furthermore the post-mortem time of appearance of giant fibres will be taken into consideration in order to compare their behaviour in chicken to that found by Remignon et al. (2000) in turkeys.

Materials and methods

A total of 11 male Ross broiler chickens were collected at the slaughterhouse just after plucking (10 minutes port-mortem) and muscle samples, parallel to fibre direction, were immediately taken from the left half of the carcasses, rolled in talcum powder, wrapped in aluminum foil, labelled, frozen and stored in liquid nitrogen until analyses were performed. The carcasses were then placed in portable coolers and taken to the laboratory where they were stored at 4°C until 24h post-mortem, when samples of the same muscles where taken from the right half of the carcasses, prepared as described above and stored in liquid nitrogen. Four muscles known, from literature and from previous studies, to have a different fibre distribution were considered: 1. *m. Pectoralis major*; 2. *m. Semimembranosus*; 3. *m.Ileotibialis lateralis*; 4. *m. Femorotibialis medius* (samples of this muscle were only taken from 5 animals). Serial cross-sections were obtained in a cryostat at -20°C and stained with hematoxylin-eosin (8µm thick) for histological evaluations and using

periodic acid schiff (PAS) (10 μm thick) for evaluation of the glycogen content. Serial, 8 μm thick, cross-sections were also processed for myofibrillar ATPase activity after acid and alkaline pre-incubation (Padykula and Herman, 1955; Guth & Samaha, 1969) and for succinate dehydrogenase (SDH) activity (Nachlas et al., 1957) or for the combination of the two (Solomon and Dunn, 1988) depending on the muscle considered. Myofibres were classified, according to the terminology introduced by Ashmore and Doerr (1971), as types βR, αR, and αW. βR fibres are stable after acid pre-incubation, labile after alkaline pre-incubation and SDH positive; both αR and αW fibres are labile after acid pre-incubation and stable after alkaline pre-incubation but while αR fibres are SDH positive, αW fibres are SDH negative.
Images were acquired and analysed, in order to determine percentages and cross sectional areas, using an image analysis system (analySIS, Soft Imaging System) implemented on a workstation equipped with a graphic card linked to a video camera placed on the microscope (Olympus BX51). Measurements were determined on about 200 fibres, from a random field in each muscle sample section stained with hematoxylin-eosin. Giant fibre percentages were calculated considering about 600 fibres from 3 random fields in order to have a better estimate of their number.
Data were statistically evaluated using Student's T test.

Results and discussion

The results concerning muscle fibre distribution of the different muscles are reported in table 1. Muscle *Pectoralis major* and muscle *Ileotibialis lateralis* are composed of mostly glycolitic fibres that have the same distribution throughout the muscle, while m. *Semimembranosus* and m. *Femorotibialis medius* are made up of various parts with a different fibre type distribution. This explains why the results are not as homogeneous for these last two muscles as they are for the first two, perhaps even a very small variation in the sampling site can determine variations in the fibre distribution.
Giant fibres did not all react in exactly the same way when they were subjected to histochemical evaluation. However, we can say that they could be classified as α type fibres as their oxidative capacity is variable yet never extremely strong, and they are more positive than α type fibres when processed for myofibrillar ATPase activity both after alkaline and after acid pre-incubation. This result is in contrast with that obtained by Chiang et al. (1995) who classified giant fibres in chicken mostly as αR and βR. Giant fibres are also always negative after PAS staining both 10 minutes and 24 hours after slaughter.
The percentage of giant fibres found in the muscle samples, 10 minutes and 24 hours post-mortem, is reported in table 2. The tables indicate that while the number of giant fibres is similar in all four muscles 10 minutes post-mortem, 24 hours post-mortem giant fibres are much more numerous in m. *Ileotibialis lateralis* and especially in m. *Pectoralis major*. The latter muscles show a significant variation in giant fibre percentage between the two sampling times ($p<0,01$) and it is interesting to notice that m. *Pectoralis major*, which has the greatest variation for this parameter, is also the muscle with the biggest difference in glycogen content between the two sampling times (table 3). This could imply that there is a link between the appearance of giant fibres and the biochemical events involving glycogen and glycolitic enzymes that take place post-mortem. This could also explain why there are usually more giant fibres in "white" muscles as seen in this experiment as well as in previous work on poultry (Chiang et al., 1995). Nonetheless, all the fibres composing these muscles are in the same condition, so it is not clear why only some of them become giant fibres. As Severini et al. (1997) suggested in a study concerning the presence of giant fibres in normal and PSE pig muscles, this could be due to a latent or very limited myopathy that could itself be accentuated by stressful conditions or by stress-susceptibility, thus explaining the higher number of these fibres sometimes seen in PSE pork. Remignon et al. (2000) in their study on turkey muscles found no giant fibres in pre-rigor muscles, while in the present study some were found. This could be due either to a species difference (chicken versus turkey) or to the small difference in sampling time (10 minutes versus 3 minutes post mortem).
Contrary to their name giant fibres are not always bigger than normal fibres. Nonetheless, on average, the mean cross sectional area of giant fibres is bigger than that of any other fibre type (tables 4 and 5).
While observing muscle samples coloured with hematoxylin-eosin, it was possible to notice that some of them contained fibres with a lower staining intensity. The evaluation and comparison of the fields containing such fibres for all of the enzymatic activities considered, proved that these fibres were always negative or only slightly positive, thus their nature and classification was not clear. These fibres resemble those found by Severini et al. (1998) in PSE pork muscle.

Conclusions

Giant fibres were more common in "white" muscles and had staining patterns similar to α type fibres. Some giant fibres also had some of the alterations usually seen in pathological muscle fibres such as an apparent hypercontraction which could explain the higher staining intensity they sometimes show. It could be said that the origin of giant fibres is connected to the biochemical events that take place during post-mortem glycolitic metabolism. There must be a reason though, that explains why only some of the fibres composing a muscle become giant fibres. This could be due to an alteration occurring in the live animal.

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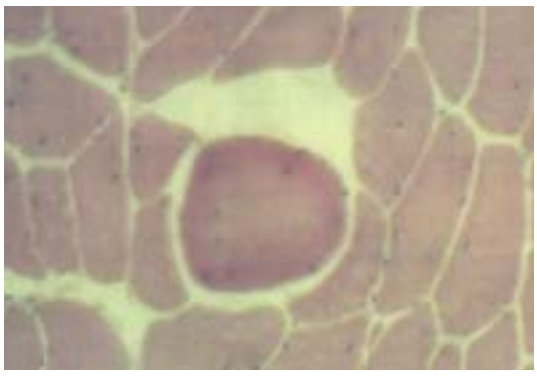

Figure 1: Giant fibre coloured with E.E.

Table 1: Mean muscle fibre distribution

	P.major	I.lateralis		Semimembranosus			F.medius		
	αW	αW	αR	αW	αR	βR	αW	αR	βR
Mean	100	71,5	28,5	62,8	33,9	3,3	58,8	40,3	0,9
St.Dev.	0	9,0	9,0	9,9	8,0	3,8	13,7	11,9	2,0

Table 2: Percentage of giant fibres 10 minutes and 24 hours post-mortem

SAMPLE	P.major		I.lateralis		Semimembranosus		F.medius	
	10 min	24 hours	10 min	24 hours	10 min	24 hours	10 min	24 hours
1	0,7	5,1	4,4	2,8	1,2	2,1		
2	0,6	3,6	1,8	4,2	1,1	3,6		
3	0,2	4,1	0,6	2,3	0,4	1,1		
4	1,0	5,1	3,8	3,8	4,5	3,4		
5	1,8	12,5	1,7	8,2	1,3	7,2		
6	0,1	7,2	0,7	2,5	0,5	3,0		
7	0,3	7,1	2,4	7,1	0,6	1,0	0,2	0,5
8	0,2	7,0	1,9	2,2	1,3	0,7	0,2	0,8
9	0,2	12,7	1,8	6,7	0,0	0,5	0,6	0,8
10	0,1	11,8	0,8	5,9	0,3	0,2	1,4	0,5
11	0,5	15,9	1,0	2,9	0,2	0,5	0,5	0,4
Mean	**0,5**	**8,4**	**1,9**	**4,4**	**1,0**	**2,1**	**0,6**	**0,6**
St.Dev.	**0,5**	**4,1**	**1,2**	**2,2**	**1,2**	**2,1**	**0,5**	**0,2**

Table 3: Amount of glycogen

Sample	P.major			I.lateralis			Semimembranosus			F.medius		
	10 min		24 h	10 min		24 h	10 min		24 h	10 min		24 h
	% positive fibres		% positive fibres	% positive fibres		% positive fibres	% positive fibres		% positive fibres	% positive fibres		% positive fibres
1	42,1	+	0	26,8	-/+	0	50,3	+/-	0			
2	66,9	+	0	49,6	+	0	5,4	+	0			
3	70,8	+/-	0	61,7	+/-	0	52,3	+/-	0			
4	79,8	+	0	15,4	+	0	23,1	+/-	0			
5	88,1	+	0	64,3	+	0	71,0	+	0			
6	87,6	++	0	62,8	+	0	70,8	+	0			
7	95,2	++	0	49,1	+	0	25,3	+/-	0	51,5	+	0
8	94,1	++	0	61,8	++	0	31,1	+	0	90,7	+	0
9	89,7	++	0	4,0	+/-	0	0,6	+/-	0	4,5	+/-	0
10	84,5	+	0	38,3	+	0	1,4	+/-	0	29,5	+/-	0
11	90,7	++	0	23,0	+	0	1,7	+	0	3,8	+/-	0

+ and – signs stand for high or low staining intensity

Table 4: Giant fibre area (μm^2)

	P.major	I.lateralis	Semimembranosus	F.medius
Mean area	5530,6	5872,3	6241,9	5478,4
St.Dev.	1342,7	1422,3	2006,2	2350,9

Table 5: Normal fibre area (μm^2)

	P.major	I.lateralis		Semimembranosus			F.medius		
	αW	αW	αR	αW	αR	βR	αW	αR	βR
Mean	3486,4	3523,0	2304,2	3231,8	2954,3	2128,0	3516,6	2251,8	1184,0
St.Dev.	1307,3	1023,5	652,9	423,1	299,5	576,9	1072,2	705,6	234,9