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NUMBER AND SIZE OF MUSCLE FIBRES IN PIGS IN RELATION TO QUANTITY AND QUALITY OF MEAT

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The elaboration and further development of the biological basis for performance testing, the search for features which correlate closely with the amount and quality of the meat and permit more precise and rapid assessment are among the foremost tasks involved in animal research.

The quality and quantity of the lean meat produced by the animal are of prime interest. The micro-structure of muscular tissue has a decisive effect on its function and quality and hence on that of the meat. It was for this reason that animal research workers exhibited increasing interest in the microscopic investigation of meat as early as the turn of the century. The results of such investigations were frequently contradictory and inconclusive although there is no lack of hypotheses regarding, for example, the connection between muscle fibres and meat quality.

The basic unit of the muscle is the muscle fibre. Muscle fibres are not actually cells but plasmodia, i.e. a multinucleate cytoplasmic mass which has developed from the cell and in which the nuclei but not the cell plasma divide. The muscle fibres are united to form bundles which are surrounded by connective tissue and, together with enclosed fat, form the most important part of the muscle tissue. The muscle fibres vary considerably in length, their length depending on the structure of the muscle. In the past, the fibre length has rarely been used to characterise the structure of meat.

The fibre diameters exhibit considerable variation and range from about 10 to 200 µm. This wide difference, which may also be observed among fibres within the same bundle, reveals the inconsistent nature of muscle tissue and the resultant difficulties encountered during microscopic investigations.

Histochemically, it is possible to distinguish between several fibre types on the basis of their enzymatic activity. The size difference between the fibre groups within the primary bundle and those at the periphery can even be observed without recourse to staining techniques. The fact that there are two muscle fibre types, i.e. light and dark fibres, has been known for some considerable time. The dark ones are also called red, type I or βfibres. They are thin, generally situated in the middle of the primary bundle, contain lipid droplets and possess a high level of oxidative enzyme activity. Similarly, the light coloured fibres are called white, type II or αfibres. Muscle fibres which occupy an intermediate position have also been described. TODOROV & PETROV (1969) reported on degenerated muscle fibres and we have also found such fibres in pigs which we investigated.Cassens et al. (1969) called them giant fibres.

SWATLAND (1972) classified muscle fibres into those ending intrafascicularly and those terminating in sinews independently of the previously mentioned fibre types.

Current opinions regarding post-natal muscle growth vary considerably, the two main theories being the following:

1. The number of muscle fibres is already fixed at birth. The muscle weight increases post-natally due to hypertrophy of the existing muscle fibres or due to transformation of the fibre type (ASHMORE, 1972)

2. The number of muscle fibres increases after birth due to the division of the existing muscle fibres, and individual large muscle fibres at the periphery of the primary bundle degenerate (BOGOLJUBSKI,1971).

This problem could be of great interest if we wish to use features of muscle fibres for predicting meat quality and meat production capacity already in pigs weighing only 40 kg.

I should now like to say a few words regarding applications for muscle fibre measurements in animal research. The best known application arises from the concept that animals with thinner muscle fibres produce more tender meat and achieve better meat quality scores. With regard to tenderness, the majority of investigations have scarcely produced convincing results since they involved animals or muscles of widely varying origins.

However, it has frequently been shown that thinner muscle fibres result in high quality meat. SCHILLING (1966) calculated a correlation coefficient of $r = + 0.47$ between muscle fibre diameter and cooking losses in hams for canning, obtained from 62 animals of identical race which received identical rations and were reared under identical conditions.

After performing his investigations, SANDOR (1971) concluded that the measurement of muscle fibres should be introduced in Hungary as an objective criterion for meat quality. DILDEY (1970) reported on the ratio between white and red muscle fibres in PSE and normal animals. The proportion of white muscle fibres was significantly higher in the PSE animals and the muscle fibre diameter was also greater on the whole. According to his investigations, the size and number of white muscle fibres are criteria for the quality of PSE meat. KLOSOWSKA (1973) noted that muscle containing a larger proportion of red muscle fibres produced better quality meat. CASSENS and co-workers (1969)investigated pigs of the same genetic origin which were reared under identical environmental conditions. Eight of the animals died during transport and were denoted stress-sensitive. The investigation of the structure of their musculature revealed a large number of giant fibres. Such fibres were scarcely found in other animals. Relations between fibre size and fibre type distribution on the one hand and the PSE meat quality and stress-sensitivity on the other have also been proved in a similar manner by COOPER (1969), ASHMORE (1972) and others.

JOUBERT's extensive investigations into growth (1958) indicated that there might be a relationship between muscle fibre thickness and the weight of the muscle. Later, TUMA (1962), LIVINGSTON (1966) and STAUN (1968) tried to predict the lean meat content of carcasses by measuring muscle fibres. For animals of the same age, the correlation coefficient between muscle fibre thickness and muscle weight varied between $r = 0.0$ and $r = 0.3$.

STAUN (1968) was able to find a closer relationship between meatiness and the total number of muscle fibres calculated from the num-

ber of muscle fibres per square millimetre (MF/mm^2) and the planimetered cross section of the M. longissimus dorsi.

Quantitative microscopic studies were commenced in the GDR on porcine muscle fibres at the Dummerstorf-Rostock Research Centre in connection with a doctorial thesis.First, a method for measuring muscle fibre characteristics was elaborated which, based on the large number of diverse preparation and measuring techniques, permits the treatment of the large number of fibres necessary due to the considerable variability of the fibres, yet can nevertheless be performed rapidly. This method was compared with two other frequently used techniques with regard to the results obtained on the same samples.

Further studies were conducted regarding the relation between muscle fibre thickness, the total number of muscle fibres and the number of giant fibres on the one hand, and selected carcass criteria characterising the meatiness and the quality of the meat on the carcass, on the other, in the case of pigs. The animals used numbered 115 and were taken from a testing station. Ninety of the animals were of the "land race" breed and 25 were various cross-breeds. The feed rations, rearing conditions and age upon slaughter were held as constant as possible.

The animals were slaughtered at the abbatoir belonging to the research centre. The carcasses were completely dissected and the normal investigations performed in the Meat Research Unit were undertaken to determine the value of the carcass.

Three muscles from the quality cuts leg, loin and shoulder were selected for the microscopic investigation: M. semembranaceus, M. longissimus dorsi and M. triceps brachii caput longum. Samples comprising about 1 cm3 each were taken at three different points of the cross section in the middle of the muscles about 24 h after slaughtering.

The following muscle fibre characteristics were determined for each sample. The diameters of 100 macerated and isolated muscle fibres were measured by means of an ocular micrometer. The complete samples were stained separately with eosin, embedded in gelatine and the cross sectional areas of the muscle fibres were determined on sections by planimetering the projected and drawn picture. The MF/mm^2 were counted using the same slides. All three features characterise the muscle fibre thickness. Furthermore, the MF/mm^2 and the planimetered cross sectional area of the M. longissimus dorsi were multiplied to obtain the total number of muscle fibres in the cross section and the number of so called giant fibres per square centimetre was determined.

A few methodological results will now be presented. After various preparation techniques had been tested, the following was found to be the best treatment of the muscle samples for performing measurements on the cross section samples: fixation for at least three weeks in 10 % formalin, staining of the complete samples in 1 % water-soluble eosin, embedding in 24 % gelatine, hardening of the gelatine to obtain a sectionable consistency by fixation in formalin or by freezing at -25^0C in a deep-freezer.

Comparison of the relative merits of formalin and Bouin's solution as fixatives showed that formalin produced the best results. Bouin-fixed samples became hard and cracked and the fixative penetrated into the reletively large samples too slowly. It was found that numerous artifacts were caused by the high temperatures involved and by dehydration in the alcohol sequence when the samples were embedded in paraffin.

Freezing experiments involving native tissues produced no satisfactory results at temperatures of -20^0C and -60^0C (mixture of dry ice and acetone). The muscle fibres either ruptured due to the formation of ice crystals or the sections rapidly disintegrated. When these investigations were performed, it had not yet become possible to freeze the samples in liquid nitrogen/ isopentane and to cut them on a cryostat microtome.

The staining of complete samples with eosin is, although less common than section staining, much quicker and completely adequate for determining the outlines of the muscle fibres. A sample manipulator which greatly reduced the amount of work involved has been developed for the proposed preparation technique (WEGNER, 1976).

Several of the numerous projection and measuring instruments described in the literature were tested: photography, the nucleus measuring instrument after Smollich, lanameter, drawing attachments and projector-type drawing mirror.

As a result, it can be said that the drawing mirror, although very simple and scarcely used, is quite suitable for muscle fibre studies.

The muscle fibre thickness in a sample can be characterised by three quantities: the muscle fibre diameter, the cross sectional area of the muscle fibre and the value MF/mm^2. We used all three methods. The best results were obtained by counting the MF/mm^2. Apart from differences between the muscles, the muscle fibre diameter revealed no significant differences or relations. Little value is attached in the literature to the determination of the diameter.

The measurement of the cross sectional area of the muscle fibre by means of a planimeter is the most precise method. However, about 40 minutes are required to planimeter 100 muscle fibres. We used the electromechanical counter "Eltinor 4" together with a "point counting rod" developed at the research centre (WEGNER, 1976) for counting the muscle fibres. This apparatus permits counting over an area of 4 mm^2 per slide in only a short time.

Because of the inconsistent nature of muscle tissue, pilot studies were conducted in order to determine the number of random samples necessary for the muscle fibre measurements. We do not subscribe to the opinion of numerous authors who regard porcine muscle tissue as homogeneous and assume that 50... 100 muscle fibres per cross section slide can be considered representative. Our studies revealed that about 1.000 muscle fibres must be measured to obtain an average value for a slide.

Comparison of the results obtained by the three methods showed clearly that greater precision is obtained by counting the MF/mm^2. We shall therefore deal only with the results obtained by this method in the following. It must be remembered that a high value for MF/mm^2 means that the muscle fibres are thin.

Table 1 shows the difference in muscle fibres according to muscle, sex and genotype. The difference between the muscles are significant, whereas the differences in case of sex and genotype are insignificant. It can be said that the M. semembranaceus has the thickest, the M. longissimus dorsi has rather thinner and the M. triceps brachii caput longum has the thinnest muscle fibres.The muscle fibres of the female crossbreeds are thicker than those of the other groups.

Table 1. Number of muscle fibres per square millimetre (MF/mm^2)

Muscle	L [1] ♂ n = 40	KR [2] ♂ n = 14	L ♀ n = 43	KR ♀ n = 14
0	1	2	3	4
M. semimembranaceus	$\bar{x}$ 264.8	248.8	263.7	228.8
	$s_{\bar{x}}$ 6.3	17.7	4.7	10.0
	s 41.2	66.3	30.7	37.3
M. longissimus dorsi	$\bar{x}$ 291.2	301.6	280.4	263.6
	$s_{\bar{x}}$ 7.9	11.7	5.5	8.7
	s 50.8	44.0	36.3	32.6
M. triceps brachii	$\bar{x}$ 411.7	410.9	401.3	340.6
	$s_{\bar{x}}$ 11.7	14.7	8.6	15.2
	s 73.0	54.9	57.3	56.7
Mean value of the three muscles	$\bar{x}$ 323.8	325.1	315.1	277.8
	$s_{\bar{x}}$ 7.4	10.2	4.9	9.8
	s 45.7	38.2	32.4	36.8

1) L - land race
2) KR - crossbreeds

Investigations revealed clear difference in fibre size between one breed and another (NESENI, 1955; OTTO, 1961).

The main object of this exercise was to study the relationship between muscle fibre thickness and the meatiness of the carcass.

Table 2 presents the calculated phenotypic correlation coefficients. They were calculated using 10 different criteria for the meatiness and the values for MF/mm^2 for the three muscles separately according to sex and pooled according to genotype. In castrated males, the correlation was lower than for the females shown here.

The negative correlations between the values for MF/mm^2 and the meatiness correspond to positive correlations between muscle fibre thickness and meatiness. The differences in the magnitudes of the correlation coefficients for the three muscles investigated can be partly ascribed to difficulties encountered in performing the measurements, particularly in the case of the M. triceps brachii caput longum.

In this muscle, differences were observed between the measuring points in the muscle itself. Even the shoulder, a cut which is allegedly representative of this muscle, is better characterized by the M. semimembranaceus.

The correlation coefficients of the order of r = 0,3 to r = 0,5 show that, in pigs of the same age , the animals produce more meat.

Table 2. Correlations between the value MF/mm^2 and the quantity of meat (sows, n = 50)

Carcass features	MF/mm^2			
	S[1]	L[2]	T[3]	$\bar{x}$[4]
0	1	2	3	4
Warm carcass weight	-0.43^{++}	-0.27^{+}	-0.16	-0.34^{+}
Valuable cuts	-0.52^{++}	-0.38^{++}	-0.14	-0.40^{++}
Protein in the half-carcass	-0.43^{++}	-0.23	-0.28^{+}	-0.38^{++}
Muscle cross sectional area	-0.51^{++}	-0.38^{++}	-0.15	-0.38^{++}
Lean meat in the leg	-0.54^{++}	-0.33^{+}	-0.10	-0.38^{++}
Protein in the leg	-0.40	-0.24	-0.07	-0.26
Lean meat in the spare rib, cutlet and fillet	-0.33^{+}	-0.17	-0.02	-0.18
Protein in the spare rib, cutlet and fillet	-0.21	-0.17	-0.14	-0.19
Lean meat in the shoulder	-0.44^{++}	-0.34^{+}	-0.07	-0.33^{+}
Protein in the shoulder	-0.34^{+}	-0.26	-0.37^{++}	-0.44^{++}

[1] S = M. semimembranaceus

[2] L = M. longissimus dorsi

[3] T = M. triceps brachii

[4] $\bar{x}$ = mean value of the three muscles

\+ $p < 0.05$

++ $p < 0.01$

Table 3 shows the correlation between the value for MF/mm^2 and the lightness of colour and water holding capacity as two quality criteria. The correlation coefficients are low and not significant. Unfortunately, our studies have not so far revealed relations between meat quality and muscle fibre, some of the causes for this being inherent in the metholological approach. For example, investigations regarding the lightness of colour, water holding capacity and muscle fibre should be performed on the same sample in the direction along the muscle fibre. Above all, however, we are of the opinion that the morphometrics of the muscle fibres alone can scarcely permit conclusions to be drawn regarding meat quality.

Some coupling between the histochemical characterisation of the different fibre types and their quantitative assessment would appear more appropriate to the problem of meat quality.

The total number of muscle fibres in the M. longissimus dorsi, a criterion proposed by STAUN (1968), is calculated from the value MF/mm^2 and the cross sectional area of the M. longissimus dorsi. Such values are presented in the next table.

Table 3. Correlations between muscle fibre characteristics and meat quality (sows, n = 25)

Meat quality criteria	MF/mm^2			Giant[1] MF/mm^2		
	S	L	T	S	L	T
	1	2	3	4	5	6
M. semimembranaceus						
colour (lightness)	-0.26			-0.18		
water holding capacity (press liquor)	-0.03			-0.10		
M. longissimus dorsi						
colour (lightness)		-0.01			-0.24	
water holding capacity (press liquor)		-0.06			-0.34$^+$	
M. triceps brachii						
colour (lightness)			-0.37			-0.37
water holding capacity (press liquor)			-0.23			-0.22

1) Number of giant fibres/cm^2

Table 4 shows the phenotypic correlation coefficients between the total number of muscle fibres and the carcass characteristics. There is a significant positive relation between the total number and the weight of the lean meat at the leg (r = + 0.3) and the loin (r = + 0.33) and a negative correlation between the total number and the amount of fat (r = - 0.28). The correlation with the water holding capacity, a meat quality criterion, is also negative (r = - 0.41). The calculations performed for castrated males produce lower correlation coefficients.

The hypothesis that a larger total number of muscle fibres in the muscle is associated with a larger quantity of meat, a lower proportion of fat and improved meat quality scores is corroborated by our results, although further results are still required with regard to meat quality scores since such studies were not the main object of our work.

Table 4. Correlations between total number of muscle fibres and carcass characteristics

Carcass characteristics	Total number of muscle fibres ♂ n = 50	♀ n = 50
0	1	2
Warm weight of carcass	0.14	0.00
Valuable cuts	0.22	0.22
Protein in the half-carcass	0.26	0.21+
Backfat (kg)	-0.04	-0.28++
Backfat in %	-0.19++	-0.37++
Muscle area	0.35++	0.52++
Lean meat in the leg	0.18+	0.30
Protein in the leg	0.30+	0.23
Lean meat in the spare rib, cutlet and fillet	0.12	0.33+
Protein in the spare rib, cutlet and fillet	0.19	0.18
Lean meat in the shoulder	0.15	0.22
Protein in the shoulder	0.27+	0.06
Colour (lightness) of the M. long. dorsi	0.25	-0.20
Water holding capacity (press liquor) of the M. long. dorsi	0.12	-0.41+

The investigations performed so far must be regarded as a basis and incentive for further research into muscle tissue. In our opinion it is necessary to find answers to a few fundamental problems regarding the development of the muscle tissue, the role played by the type of fibre, the internal morphological structure of the muscles and to questions involving post-mortem changes in the muscle fibre in order to be able to work more precisely during the sampling and measuring stages.

In connection with the treatment of samples, the aim must be to maintain the structure with as little modification as possible. Modern techniques such as deep-frozen cutting using cryostats indicate the way. The application of modern automatic micro-picture analysers is essential for the rational evaluation of the samples required to characterise the musculature of an animal and in view of the large numbers of animals involved. From the literature and the results presented here it must be concluded that the quantitative microscopic investigation of muscle, as the place in which meat as a criterion for the performance of the animal is produced, is absolutely necessary both for fundamental research in connection with animal research questions and for meat research as a more practical aspect.

In this connection, the investigation of the muscle is on no account to be restricted to the morphology of the muscle fibres.

Histochemistry and electron microscopy, as well as biochemical and biophysical disciplines must cooperate closely to find characteristics and new, more rapid and precise selection criteria both for muscular growth, and thus for the increase in lean meat, and for the quality of the meat.

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