Determination of the origin of poultry breast meat

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Introduction:

In the Netherlands the consumption of further processed poultry meat products has increased in the last 5 to 10 years. In that period products like marinated broiler breast meat fillets, schnitzels, etc. were produced and sold in large quantities. For quality assurance reasons the Dutch poultry industry is preparing legislation for these products. Especially the breast meat used in these products should be from broiler type chickens if declared as such and not from spent hens. Such a demand asks for a proper method for the determination of the origin of breast meat.

A possible parameter for the differentiation is the fibre size of the breast muscle. Aberlee et al. (1979) found differences between fibre size of M. sartorius in 4 weeks old birds (17.1 µm in broiler, 14.2 µm in layer). Smith (1963) also showed fibre size differences in broiler and layer type poultry.

The number of fibres in a muscle is determined before hatching and the increase in muscle cell size occurs primarily during the post hatching period. Smith found a larger number of cells in the broiler type bird of almost the same size at the same body weight. When the birds were heavier, the broiler type bird showed larger muscle fibre cells.

Figure 1 shows the M. sartorius cell diameter at different ages from a layer type bird and a selected fast growing type (after Smith, 1963).

At the same age the muscle cell diameter of the different types of birds showed remarkable differences. The fibres of broiler type muscles were found to be larger then the fibres of the layer type birds.

However, normally broilers are slaughtered at the age of 6 or 7 weeks and spent hens are normally slaughtered after one laying period at the age of about 70 weeks or more. When comparing the fibre sizes of the broiler type birds at 6 weeks of age with the layer type birds at 35 weeks of age, the latter have larger diameters. This difference can even become larger at a higher age of the hens.

Uhrin and Kuliskova (1984) found larger differences in fibre size of the M. pectoralis major when compared with the M. iliotibialis posterior between broiler type birds and spent hens. The results suggest that the fibre size of the M. pectoralis major can be used as a criterion to determine the origin of the breast meat used in the further processed breast meat products.

Materials and Methods:

Histology

Method I: Trichloroacetic acid (TCA) fixation, cross sections.

From the M. pectoralis major a piece of 1x1x2.5 cm^3 is taken following the direction of the fibres. This piece of muscle is fixed by needles onto a slice of cork. The tissue is then submerged into the fixation fluid (16% TCA solution).

After 3 hours the needles and the cork are removed and the fixation is continued for about 48 hours. Then the muscle can be dehydrated and embedded in paraffine. Dehydration and embedding takes place by successive treatment of the samples with ethanol 96%, ethanol 100%, methylbenzoate, xylol and paraffine. Then microtome sections with a thickness of 4 microns taken at right angles on the fibre direction, are made. The sections are stretched and attached to an object glass and dried for 24 hours at 40°C. After removing the paraffine with xylol and washing with ethanol the sample is coloured with an 1% eosine solution in 96% ethanol and Mayer's haemalum. Then the sample is dehydrated again with ethanol and xylol respectively. From 2 or 3 places of the sections microscopic Polaroid photographs are made (enlargement about 160x). In each photo the area of 24 muscle fibres is measured with an electronic planimeter.

Method II: Formaline fixation, cross sections

This method is identical to method I except for the following steps:

- Fixation with 10% formaline pH 7.0
- Removing of the needles and the cork takes place after 6 hours
- Duration of the fixation 5 x 24 hours
- Dehydration occurs with ethanol 70%, ethanol 80% (2 times), and ethanol 96% respectively.

Method III: TCA fixation and maceration of the muscle pieces

From the M. pectoralis major a piece of muscle is cut and submerged into a 16% TCA solution until the connective tissue has loosened. From this piece of muscle 0.1 g is stirred in 16% TCA and centrifuged during 2 minutes at 2000 rpm.

This procedure of stirring and centrifuging is repeated with respectively ethanol 96% (2 times), eosine solution (1% in ethanol 96%), ethanol 100% (2 times), ethanol 100%/xylol 1:1 (2 times) and xylol (2 times).

Finally the suspension of free muscle fibres is mixed with entellan and some drops are put upon an object glass slide. From this suspension photographs are taken (enlargement about 40x) with a Polaroid 667 film. The diameter of the individual muscle fibres is measured from these photos by hand.

Experiment 1

M.pectoralis major of 2 birds from 4 broiler flocks at the age of 6 weeks, 3 layer flocks and 1 broiler parent flock was sampled. Samples of meat were taken and the mean fibre size of 72 fibres was estimated with all three methods.

Experiment 2

To obtain further information about the effect of the sampling location samples were taken at the cranial and caudal end and of the midpoint of the M. pectoralis major. Two flocks of each broiler, spent hens and broiler parents were sampled. Method I was used to estimate the mean fibre size of 72 fibres.

Experiment 3

The way of processing of the breast meat could also influence the fibre size. The breast meat of each 3 flocks broilers, spent hens and broiler parents was treated in the following ways:

- 1. Removing of the meat from the carcass directly after killing and bleeding
- 2. As 1, but the meat remained attached to the bone

- 3. Meat from industrially slaughtered birds, removed from the carcass after cooling
- 4. As 3. The meat was then frozen and thawed before sampling
- 5. As 3. The meat was then stored in an 1% NaCl solution at 0°C during 24 hours
- 6. As 3. The meat was then tumbled in a marinade and stored during 16 hours at 0°C

After the given treatments the meat was sampled to estimate the mean fibre size of 48 fibres per muscle (Method I).

Results and Discussion:

Experiment 1
The results of experiment 1 are given in table 1. The data show clear differences between broilers, spent hens and broiler parents. The different histological methods used give somewhat different results but the same trend is found with each of the methods used. These differences can be explained by the way of treatment during fixation which can cause swelling or shrinking of the muscle fibres.
As the TCA fixation gives the possibility to use the samples to produce cross sections or to obtain macerated fibres this method was used in the other experiments. Also the fixation time of the TCA fixation method is shorter.
The method to produce macerated fibres is still under study but the time needed to obtain complete macerated fibres varies from 2 to many weeks. Further study is required to know exactly how to make the maceration process quicker (< 2 weeks).
Figure 2 gives some photographs of cross sections of muscles and macerated muscle fibres of the different categories of birds. Especially the cross sections show here the differences in fibre size quite clearly.

Experiment 2
The results of the fibre size measurements of experiment 2 are given in table 2. No significant effect could be found of the location of sampling within the muscle. This means that if the method is to be used to determine the origin of the meat in breast meat products with small pieces of meat, the method is also applicable.

Experiment 3
Treatment of breast meat during and after slaughtering of broilers, spent hens and broiler parents does not cause any significant effect on the fibre size. Neither the other parameters in this experiment, the flock and the location of measurement in the sample, nor any interaction had significant effects. Only the type of bird (broilers, spent hens and broiler parents) had highly significant effects.
The data of this experiment are given in figure 3 together with all the data obtained with method I from experiment 1 and 2.
Figure 3a gives the frequency distribution of all the individual data from the experiments 1, 2 and 3.
It is evident that, based on these results, it is not possible, to decide whether a piece of breast meat originates from spent hens or from one of the other types because of existing overlaps.
When based on the mean value of 2 or 3 randomly chosen breast meat samples from experiment 1, 2 and 3 frequency distributions as given in figures 3b and 3c respectively are obtained.
This illustrates that it is possible to distinguish between broilers and spent hens or between spent hens and broiler parents based upon the muscle fibre size when enough (minimum 3) breast meat samples are analysed.
Some reservations must be made because of the limited number of measurements, done sofar. Further analyses will be carried out.

Literature:

Aberlee, E.D., Addis, P.B. and Shoffner, R.N. (1979). Fibre types in skeletal muscles of broiler- and layer-type chickens. Poultry Sci. 58: 1210.
Uhrin, V. and Kulísková, L. (1984). Histologicka a histochemicka stavba vicktorych svalov hydiny. Potravinarske vedy 2(20): 171.
Smith, J.H. (1963). Relation of body size to muscle cell size and number in the chicken. Poultry Sci. 42: 283.

Table 1. Mean fibre size of breast meat of broilers, spent hens and broiler parents with different histological methods.

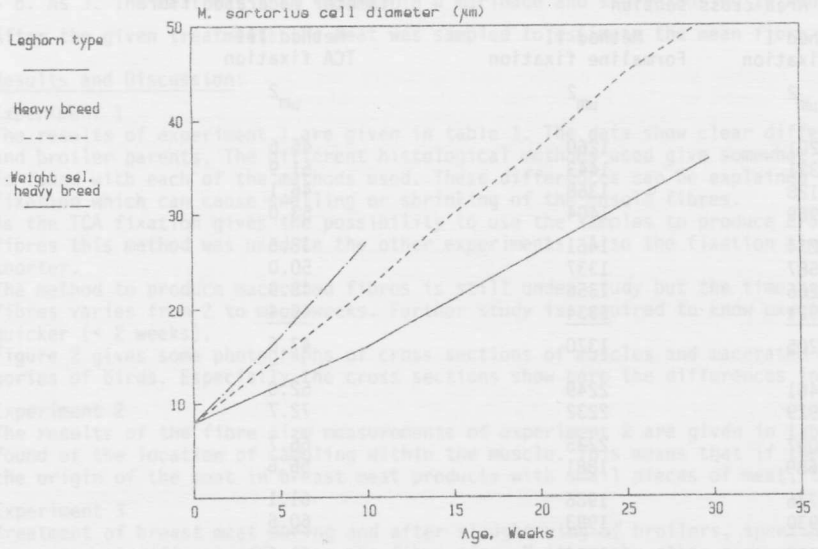
			Area cross section		Diameter macerated fibre
			Method I TCA fixation	Method II Formaline fixation	Method III TCA fixation
		Flock	μm^2	μm^2	μm^2
Broilers	Hybro	1	1201	1260	45.6
		2	1331	1443	51.9
		3	1128	1368	46.1
		4	969	1404	53.0
	Hypeco	1	1234	1461	38.6
		2	1587	1337	50.0
		3	1266	1356	48.9
		4	921	1334	46.4
	$\bar{x}$		1205	1370	47.6
Spent hens	Warren	1	2461	2249	52.0
		2	2929	2232	72.7
	Shaver	1	2097	2235	66.7
		2	1689	1881	56.6
	Hisex	1	1736	1986	61.1
		2	2930	1983	60.6
	$\bar{x}$		2307	2094	61.6
Broiler parents	Hypeco	1	3029	2905	91.4
		2	2829	2650	81.6
	$\bar{x}$		2929	2777	86.5

Table 2. Mean fibre size of breast meat of broilers, spent hens and broiler parents at different sampling places.

	area cross section muscle fibres (μm^2)					
Sampling place	broiler		spent hen		broiler parent	
	1	2	1	2	1	2
1	1740	1553	2342	1795	2772	3016
2	1671	1736	2200	1952	3156	3196
3	1886	1557	2036	2261	3444	3152

Figure 1

Cell diameter of M. sartorius



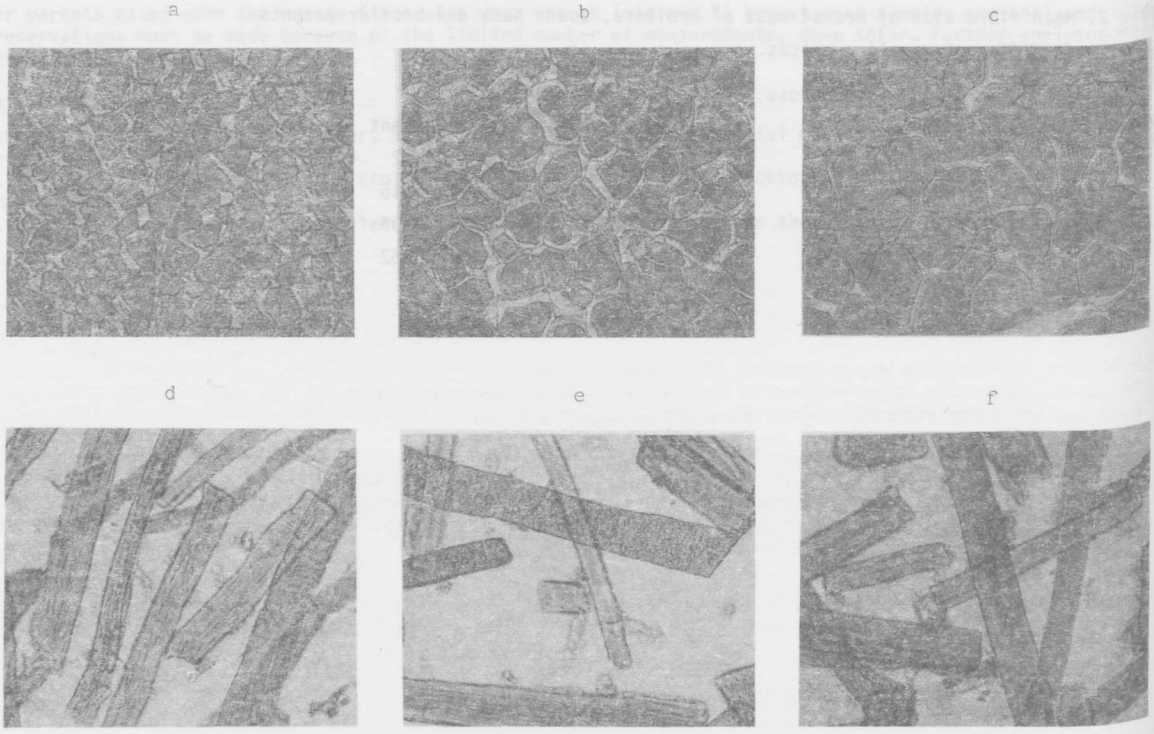


Figure 2. Cross section and macerated fibre photographs of breast meat from broilers (a,d), spent hens (b,e) and broiler parents (c,f). TCA fixation.

Figure 3

Frequency distribution of the mean fibre size of individual breasthalves (a), and of the mean value of 2 (b) and 3 (c) randomly choosen breasthalves from broilers, spent hens and broiler parents.