INFLUENCE OF MUSCLE LOCATION ON FATTY ACID COMPOSITION IN THE PIG

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Introduction

A hyperbolic relationship between the percentage of fat in animal tissues, and the iodine number of the fat, was described by Callow and Searle (1956). They explained it on the basis that the structural lipid elements of the cell, which contain a substantial proportion of fatty acids with several double bonds, were diluted by saturated fatty acids - mainly synthesized from dietary carbohydrate - when increasing quantities of fat were deposited. A plot of the mean iodine number against the percentage of intramuscular fat for some thirty samples of each of eight porcine muscles (Lawrie, Pomeroy & Cuthbertson, 1963) indicated, however, that there were substantial deviations from such a relationship. It thus appeared that different muscles might well have characteristically different fatty acid and phospholipid components, reflecting their functional specialization *in vivo*; and reflected by their nutritional and organoleptic quality as fresh meat as well as by their behaviour in storage. It also seemed feasible that there might be differences between muscles in the distribution of the lipids between various functional divisions - the contractile fraction (myofibrils), the fraction concerned with linking oxidation to energy production (mitochondria) and the regulatory fraction (microsomes).

The present paper reports some preliminary findings from an investigation in which the effect of animal age and diet on the fatty acid composition of neutral lipids and phospholipids in different porcine muscles is being studied. The data here presented

refer only to the total lipids from nine locations in an adult pig.

It is clear, however, that even when died and age are constant, the anatomical location influences the pattern of fatty acids on porcine muscle.

Methods. The muscles chosen for examination were removed from the chilled carcase within 24 hr. of death. Each muscle was freed from extraneous fat and connective tissue, wrapped in aluminium foil, dipped in liquid nitrogen and stored in vacuo at -20°C.

The anatomical areas examined were as follows :- (I) l.dorsi: level of 4th - 6th lumbar vertebrae, (II) l.dorsi: level of 13th - 15th thoracic vertebrae, (III) l.dorsi : level of 8th - 12th thoracic vertebrae, (IV) l.dorsi: level of 5th - 7th toracic vertebrae, (V) semimembranosus: entire, (VI) rectus femoris: entire, (VII) psoas: entire, (VIII) diaphragm: entire, (IX) supraspinatus: entire. The muscles chosen were regarded as representative of a substantial, and commercially important, portion of the pig carcase.

The method used for extracting total lipids followed that of Bligh & Dyer (1959). Methyl esters of the fatty acids in the lipid extracts were prepared by the procedure of the American Oil Chemists Association (Anon., 1966). The solidified esters were dissolved in 2:2:4-trimethylpentane at a conc. of 100 mg/ml; and stored at -20°C prior to gas liquid chromatography.

To obtain the different functional fractions, 100 g muscle were homogenized in 4 vol. ice-cold 0.1M KCl, containing 5mM histidine, pH 7,2, for 2 min. and centrifuged (Martonosi & Feretos, 1964) at 0°C as follows :-

Fraction	Centrifuging Conditions	
	g	min
myofibrils	1,000	20
mitochondria	8,000	60
Grana I (heavy microsomal fraction)	30,000	60
Grana II (light microsomal fraction)	60,000	60

The fractions were suspended in 200 ml. 0.1M KCl: 5mM histidine and stored at 0°C prior to lipid extraction and methylation as above.

GLC of the fatty acids prepared from the lipids of whole muscle, and from the various fractions, was carried out using an Aerograph Hy-Fi 600C machine. Columns of polyethylene glycol adipate and diethylene glycol succinate were employed. The latter gave better resolution of closely related species (e.g. palmitic and palmitoleic). Fatty acid esters were injected into the column in2:2:4-trimethyl pentane, (100mg/ml). GLC runs were carried out isothermally at 175°, 185° and 190°C. Peak identification was made by reference to mixtures of pure fatty acid methyl esters.

Results

The percentage of total lipid, and of triglycerides in the lipids, together with the relative concentrations of the fatty acid constituents detected in the total lipids at the 9 anatomical sites examined, are represented in Table 1. Apart from expectable variabilities in fat content between the sites, it is evident that there is some variation in the percentage of triglyceride present. The low values of the latter in diaphragm, psoas and l.dorsi (thoracic 8-12) connotate a correspondingly higher content of phospholipid than is present elsewhere. It is apparent that the general pattern of fatty acids at all sites studied is

similar, namely, in decreasing order of abundance, oleic (C18:1), palmitic (C16:0), linoleic (C18:), stearic (C18:0) and palmitoleic (C1-:1). There are, however, some noteworthy variations in detail. The content of palmitic is relatively high in l.dorsi (thoracic 8:12), that of linoleic in psoas and rectus femoris and that of stearic in supraspinatus. In respect of minor fatty acid components, however, variation between sites is more marked (Table I (II)). The apparent absence of arachidonic (C20:4) from supraspinatus and its relatively low concentration in diaphragm and l.dorsi (lumbar 4-6) is of interest, as is the variation in the contents of fatty acids having uneven numbers of carbon atoms in the chain (C15:1, C17:1 and C13:0).

The distribution of major fatty acids in the total lipids extracted from various functional fractions from l.dorsi (thoracic 8-12), rectus femoris and supraspinatus is shown in Table 2. The high percentage of total lipid in the two microsomal fractions (especially in the heavy microsomal fraction, grana I, which has the greater Ca^{++} - accreting ability of the two: Martonosi & Feretos, 1964), and its apparently predominant phospholipid character, is evident. It may be observed, also, that variability in the fatty acid pattern between the muscles is more marked in mitochondrial and microsomal fractions than in the myofibrils. (The pattern for the latter would be anticipated to ressemble that of the unfractionated muscle (Table 1) by virtue of the predomiant contribution of the myofibrils to the bulk of the whole muscle). In comparing the mitochondrial patterns from the three muscles, the low content of palmitic acid, and the high content of linoleic acid, in those from rectus femoris may be noted. While Grana II - the heavy microsome fractions - have a more uniform pattern bet-

ween the three sites (notwithstanding the relatively high content of oleic, and the low content of linoloic, in supraspinatus) intermuscular variability is particularly striking in respect of all five major fatty acid components of the total lipids extracted from the microsomal fractions.

Discussion

The general pattern for the relative percentage of the major fatty acids in the lipids of porcine muscles presently reported - namely, oleic, palmitic, linoleic, stearic and palmitoleic, in descending order - accords with the findings of Allen, Bray and Cassens (1967). These workers studied l.dorsi only (at the level of the 2th - 5th lumbar vertebrae) in Duroc boars, gilts and barrows: their data for the neutral lipid fraction of this muscle correspond very closely with those for l.dorsi (lumbar) in Table 1 (b). On the other hand, Mason & Sewell (1967) found that stearic and palmitoleic were respectively the second and third most prevalent fatty acids in the lipids of l.dorsi in the adult pig. The breed was not specified, however, and this may account for the different pattern.

Although fatty acids containing an odd number of carbon atoms are relatively rare in animal tissues, they have been reported in phospholipid fractions (Peng and Duggan, 1965). Traces of C17:0 found in the present investigation may well have been dietary in origin since this fatty acid was found in the molassine meal of the feed. It has hitherto been reported in lard (Magidman, Hart, Luddy and Riemanschneider, 1963) and in bovine intramuscular lipid (Hornstein, Crowe & Hiner, 1968 : Terrell, Suess, Cassens and Bray, 1968). Despite their lower absolute concentration, the rather larger differences between the muscles studied in respect of this

minor fatty acid components may well have considerable significance in determining their relative properties.

It is of interest that Krzywicky & Ratcliff (1967), in a study of porcine l.dorsi found that while the ratio of the different types of phospholipid was similar at 1st and 6th lumbar and 7th thoracic levels, the total phospholipid phosphorus was markedly gigher at the first of these locations than at the other two.

Although differences in lipid composition between overtly 'red' or 'white' muscles might have been anticipated as a reflection of their differing capacities for respiratory metabolism (Lawrie, 1953 : Gauthier and Padykula, 1966), the distinctions here found re-enforce those from protein analysis (Parsons, Parsons, Blanshard and Lawrie, 1969) in indicating a greater measure of complexity in muscle differentiation than the classification of 'red' or 'white' would suggest. It must be presumed that these differences, in turn, will be reflected in the organoleptic, nutritive and keeping attributes, as meat, of the various muscle locations.

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Table 1. Relative fatty acid composition of total lipids from various porcine muscles of 3 year old pig

Muscle	Total lipid (% wet wt.)	Triglyceride (% total lipid)	Fatty Acids (as % total fatty acids)									
			(i) major					(ii) minor				
			C18:1	C16:0	C18:2	C18:0	C16:1	C20:4	C14:0	C15:1	C17:1	C13:0
diaphragm	4.5	50.9	35	22	15	15	5	2.0	1.5	1.0	0.8	*
psoas	1.6	54.0	33	22	17	12	5	3.8	1.2	2.3	1.5	*
semimembranosus	1.1	58.5	35	24	14	10	4.5	4.2	1.5	2.5	1.3	0.3
l.dorsi (lumbar 4-6)	1.9	66.9	40	24	11	10	5	2.5	1.5	1.5	0.8	*
l.dorsi (thoracic 13-15)	1.1	71.0	37	21	12.5	11	5	4.8	1.0	3.2	1.2	*
l.dorsi (thoracic 8-12)	3.1	52.5	35	30	11	10	3.5	4.3	1.0	2.5	0.5	*
l.dorsi (thoracic 5-7)	1.2	59.0	38	21	13.5	10	4	4.2	1.5	2.5	1.0	0.2
rectus femoris	3.2	60.0	29	21	16	11	4.5	5.8	1.0	3.7	0.5	0.2
supraspinatus	3.6	76.6	34	26.5	14	19	6	*	*	4.8	*	*

* not detectable

Table 2. Distribution of major fatty acids in total lipid of various fractions from three porcine muscles of three year old pig.

Fraction	Muscle	Wt. Fraction as % muscle wt.	Total lipid in fraction (%)	Neutral lipid as % total lipid	Major Fatty Acids (as % total fatty acids)				
					C18:1	C16:0	C18:2	C18:0	C16:1
Myofibrils	l.dorsi (thoracic 8-12)	66.8	1.6	58.9	32.3	23.9	14.1	9.8	4.5
	Rectus femoris	60	1.5	30.0	33.5	22.3	18.4	7.4	3.8
	Suprasplnatus	47	2.8	33.1	34.5	22.5	16.8	10.9	4.0
Mitochondria	l.dorsi (thoracic 8-12)	0.8	5.1	42.5	39.0	26.2	6.3	11.3	8.9
	Rectus femoris	0.2	20.6	33.6	45.5	5.8	11.2	12.6	8.1
	Suprasplnatus	1.8	3.6	54.9	25.6	17.9	8.6	8.8	4.0
Grana I	l.dorsi (thoracic 8-12)	0.5	77.5	1.4	23.9	20.2	10.7	11.3	5.3
	Rectus femoris	0.5	98.2	1.5	25.6	19.2	17.1	13.6	10.5
	Suprasplnatus	0.4	96.5	2.1	34.8	23.7	8.4	11.2	5.0
Grana II	l.dorsi (thoracic 8-12)	0.3	63.8	12.8	18.1	21.7	2.0	12.2	12.0
	Rectus femoris	0.4	55.3	6.1	47.4	52.6	*	*	*
	Suprasplnatus	0.3	50.2	4.3	35.2	27.5	19.8	14.3	*

* not detectable