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Sex And Weight Associated Changes In Fatty Acid Composition Of Porcine Muscle Lipid

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SUMMARY

The fatty acid composition of porcine longissimus dorsi muscle lipid from 30 animals slaughtered at two live weights (49.6 and 94.1 kg.) and consisting of an equal number of boars, barrows and gilts was determined. The lipid was separated into the neutral lipid and phospholipid fractions prior to analysis.

Odd-numbered fatty acids C11, C13, C15 and C17 were significantly higher in the phospholipid fraction. Fatty acid C17 was never detected in the neutral lipid fraction.

Both weight and sex effects were quite restricted to the neutral lipid fraction. Weight had a significant effect on fatty acids C10, C11, C12, C14, C16 and C18 in one or more of the sexes. The most interesting result was the marked increase in fatty acid C18 of the heavy weight (94.1 kg.) gilts and barrows as compared to its very constant level in the muscle from boars of both weights. This difference coupled with significantly lower C18-1 values in heavy weight barrows and gilts when compared to boars resulted in a markedly higher mean ratio of fatty acid C18:1 to C18 for heavy weight boars. Thus these data suggest that much of the increase in saturation of intramuscular lipid that occurs after about 55 kg. live weight can be attributed to the ratio of fatty acid C18:1 to C18. Furthermore this change is dependent

upon sex hormones and is restricted to the neutral lipid fraction.

INTRODUCTION

Interest in the muscle lipids of porcine animals is related to their contribution to the organoleptic properties of meat (Younathan and Watts, 1960; Kauffman et al., 1964). The studies of Hornstein et al. (1961), and Giam and Dugan (1965) have been on the fatty acid composition of porcine intramuscular lipid. While it is known that factors such as ration Chung and Lin (1965) can have marked effects on lipid composition, the effects of other variables such as age and sex are less well-known. Although Lawrie et al. (1963) found no difference in iodine number of porcine intramuscular lipid from animals of 150 and 250 pounds live weight, other work (Elson et al., 1963; Allen et al., 1964b) indicates that a marked decrease in iodine number of intramuscular lipid occurs sometime after approximately 125 pounds live weight. In addition, Sink et al. (1964) reported a general increase in the saturated fatty acids of porcine subcutaneous lipid at a similar stage of maturity. Dahl (1958) attributed such increases in lipid saturation to the more saturated energy source of older animals. However, the results of Sink et al. (1965) using radiochlorine labelled fatty acids suggest that some metabolic changes are occurring in the subcutaneous lipid depot of porcine animals at about 125 pounds live weight.

The fact that barrow carcasses generally contain more fatty tissue than carcasses from gilts (Charette, 1961; Allen and Bray, 1964a) or boars. (Lidvall et al., 1964) suggests that sex may also exert an influence on fatty acid composition. The data of Lawrie et al. (1964) for iodine number of intramuscular lipid from boars and hogs, suggests that differences in fatty acid

composition exist between intact and castrated males.

The purpose of this study was to investigate the influence of sex (boars, barrows and gilts) and live weight in porcine animals on the fatty acid composition of the longissimus dorsi muscle lipid. Two fractions, the neutral lipid (chloroform soluble) and phospholipid (methanol soluble) were studied.

METHODS

A total of 30 Duroc animals consisting of one boar, one barrow and one gilt from each of ten litters were allotted equally to two slaughter groups, light weight (49.6 kg.) and heavy weight (94.1 kg.). All animals were maintained on the same ration (ad libitum) under similar conditions until they reached the respective slaughter weight for their group. They were then fasted for approximately 16 hr, exsanguinated and a 70-100 g sample of the left longissimus dorsi muscle was excised dorsal to the 2nd to 5th lumbar vertebrae. This muscle sample was trimmed free of all extramuscular fat, placed in a plastic bag, frozen immediately in liquid nitrogen and stored at -20°C for 10-30 days. Upon removal from the freezer, 50 g of the still partially frozen muscle sample was diced and extracted with chloroform-methanol as outlined by Ostrander and Dugan (1960). After filtering the slurry under an atmosphere of carbon dioxide, phase separation was allowed to proceed at room temperature for 2-3 hr in a separatory funnel. Following phase separation, the volume of chloroform was recorded and two 5 ml aliquots of chloroform were evaporated and dried at 100°C for 10-12 hr. The weight of residue after drying was used in determining the percent of lipid in the muscle sample. The remainder of the chloroform

phase was transferred to an Erlenmeyer flask, covered with nitrogen and stored at 5°C until used for the lipid fractionation 1-2 days later.

Separation of the neutral lipid and phospholipid fractions was according to the procedure of Choudhury and Arnold (1960) with the following modifications. Silicic acid (2 g) activated at 120°C for 30 min and 110-130 mg of lipid were shaken with 25 ml chloroform for 20 min. Prior to filtering the lipid-chloroform-silicic acid mixture, approximately 0.5 g of dry, activated silicic acid was placed in the sintered glass filter. Filtration of the above mixture was then carried out and the silicic acid was washed with 100 ml of chloroform to elute the neutral lipids. Prior to washing the silicic acid with methanol, care was taken to rinse the bottom and sides of the filter with chloroform to remove traces of neutral lipid. Approximately 10 ml of methanol was then passed through the filter into the suction flask, recovered and transferred back into the filter. The silicic acid was then washed with 80 ml of methanol for isolating the phospholipid fraction. Thin-layer chromatography and subsequent exposure of the developed plate to iodine vapor for spot identification indicated no cross contamination in either fraction.

The neutral lipid and phospholipid fractions were concentrated on a vacuum evaporator and all of the phospholipid fraction and approximately one-third of the neutral lipid fraction was transferred to a 20 ml glass-stoppered centrifuge tube. The lipid was then taken to dryness with a rotary evaporator and 1 ml of benzene was added. Following this, 8 ml of a 1% sulfuric acid-super dry methanol solution and approximately 0.5 bm of anhydrous sodium sulfate were added to the test tube. The contents were blanketed with nitrogen, stoppered and placed in a 60°C water bath for $2\frac{1}{2}$ hr with

periodic mixing. The methyl esters were then isolated according to the procedure of Sink et al. (1964). It should be noted that all procedures were conducted in either a carbon dioxide or nitrogen atmosphere, including the release of a vacuum in the evaporating steps.

Gas-liquid chromatography of the methyl esters was conducted on a dual column, Aerograph model 204 Gas Chromatogram using dual flame ionization detectors. A coiled glass column, 8 ft long and 1/8 in. in diameter, was packed with 15% diethylene glycol succinate (DEGS)o on Chromosorb W at 45 psi. The column oven was maintained at 185°C and the flow rate of nitrogen gas was 22-24 ml/min. The injector and detector temperatures were 220°C. The methyl esters of the fatty acids from the neutral lipid and phospholipid fractions of the same sample were run simultaneously, one on each column. The individual fatty acid peaks were identified by comparison of retention times to those in a known standard of similar composition and concentration. In addition semilogarithmic plots of carbon number vs. relative retention time were used for identification. The fatty acids were quantitatively evaluated by the peak height method of Baumgardt (1964) using a standard run on the same day as the sample. All fatty acids are reported as weight percentages of the total known fatty acids present. Statistical analyses performed are described by Steel and Torrie (1960).

RESULTS

The data (Table 1) for weight effect have been analyzed on a weight/fraction/sex basis. These data show that live weight had little effect on the fatty acid compostion of the phospholipid fraction of lipid from porcine longissimus dorsi muscle. However a number of fatty acids in the neutral lipid

fraction were influenced by slaughter weight of the animal. Results for fatty acids C10, C11, C12 and C18 of the neutral lipid fraction show that they were significantly higher in one or more of the heavy weight as compared to the light weight groups. Fatty acids C14 and C16 were shown to be significantly lower in two sexes of the heavy weight group. Quantitatively the most interesting change that occurred with an increase in live weight was the increase in fatty acid C18 in the barrows and gilts, while remaining at a similar concentration in boars of both weights.

The difference in response of C18 in the heavy weight sex groups resulted in significantly higher C18 fatty acid values in the neutral lipid fraction for barrows and gilts as compared to boars (Table 2). The other quantitatively important sex difference was for C18-1 where in the heavy weight group barrows and gilts had values significantly lower than those for boars.

Sex (Table 2) had no significant effect on fatty acid composition in the phospholipid fraction of either weight group. There were three significant (C14, C18 and C18-1) sex-weight interactions as shown in Table 2.

Results in Table 1 show a most interesting distribution of the odd-numbered fatty acids C11, C13, C15 and C17 between the two fractions for all sex and weight groups. These data show that odd numbered fatty acids were more predominant in the phospholipid fraction. Fatty acid C17 was never detected in the neutral lipid fraction at the sensitivities used in this experiment.

It should be noted that three additional fatty acid peaks were detected in most phospholipid chromatograms but are not included here because standards were not available for their quantitation as carried out for these

data. Their retention times relative to C18 were 0.45 (X1), 0.86 (X2) and 3.13 (X3). Plots of log retention time against carbon number indicated that they were most likely C14-2 (X1), C16-2 (X2) and C22 (X3) fatty acids and were estimated to make up approximately 5.0, 0.8 and 1.0%, respectively of the total fatty acids in the phospholipid fraction.

Data in Table 3 show that the intramuscular lipid content was not significantly different between sexes even though boars tended to have a lower mean value. Weight however had a significant effect on intramuscular lipid content in all three groups.

DISCUSSION

The results of this study indicate that much of the increase in saturation of porcine intramuscular lipid occurring after approximately 55 kg live weight (Elson et al., 1963; Allen et al., 1964b) can be accounted for by the increase in fatty acid C18 and a decrease in fatty acid C18-1. Furthermore this change has been shown to occur in the neutral lipid rather than the phospholipid fraction. It is interesting that the weight effect on the ratio of C18-1/C18 was present in the longissimus dorsi muscle lipid of barrows and gilts but not boars. Thus these data suggest that the higher iodine number of boars when compared to barrows (Lawrie et al., 1964) is largely determined by the quantities of C18 and C18-1 in the neutral lipid fraction.

Since there was no significant difference between sexes for intramuscular lipid content, it is doubtful that the fatty acid differences (Table 2) were due to lipid content rather than sex. These data show that the weight effect (Table 1) on fatty acid compostion was concomitant

with significant increases in intramuscular lipid content of all three heavy weight groups. Thus the boar data (Table 1 and 3) suggest that increases in intramuscular lipid content are not always attended by increases in lipid saturation.

These data give additional support to the findings of Cramer and Marchello (1964) in ovine animals that a certain level of maturity is necessary before the sex hormones are effective in altering fatty acid composition. The reason for a sex effect may in part be explained by the histochemical enzyme data on these porcine muscle samples (Allen et al., 1966). Those data show that boars when compared to gilts and barrows have a larger number of muscle fibers with the enzymatic capacity to mobilize and oxidize lipid. This was most evident in the heavy weight group. One would expect the androgens of the boar to stimulate lipid catabolism, which could alter fatty acid composition through preferential fatty acid oxidation (Rothlin and Bing, 1961; Bollinger, 1964). Investigations with humans (Havel et al., 1963) and dogs (Issekutz et al., 1964; Spitzer and Gold, 1964) have suggested the important role of intramuscular lipid as an energy source. As another possible mechanism which could contribute to the sex differences for fatty acids C18 and C18-1, it would seem reasonable to suggest that the sex hormones influence the desaturase enzyme (Marsh and James, 1962) which converts C18 to C18-1.

Magidman et al. (1963) have reported the presence of fatty acid C17 in lard. In addition Peng and Dugan (1965) found that fatty acids C11, C13 and C17 occurred in the muscle tissue of chickens and in some instances were specific to certain phospholipids and dark or white muscles. The results of this study show that the majority of the odd numbered fatty

acids occurred in the phospholipid fraction, regardless of sex group or light weight. The reason for such a distribution poses an interesting question.

In conclusion it seems especially pertinent that the influence of sex and weight on intramuscular fatty acid composition were essentially restricted to the neutral lipid fraction with little or no effect occurring in the phospholipid fraction. This is consistent with the concept of two fatty acid pools existing in tissues as suggested by labelling experiments of Volk et al. (1952) and Coniglio et al. (1964). The fatty acid pool which incorporates label more slowly is associated with the cell membranes (phospholipids) and is therefore less subject to change. The other pool which is more rapidly labelled consists of the unbound fatty acids (neutral lipid) and is in continual flux.

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Table 1. Effect of weight on fatty acid composition of neutral lipid and phospholipid fractions[a]

Fatty acid No.	BOARS						GILTS						BARROWS					
	Fraction 1[b]			Fraction 2[c]			Fraction 1			Fraction 2			Fraction 1			Fraction 2		
	Lt.[d]	Hvg.[e]	F	Lt.	Hvg.	F	Lt.	Hvg.	F	Lt.	Hvg.	F	Lt.	Hvg.	F	Lt.	Hvg.	F
10	.08	.13	*	.11	.14	ns	.12	.13	ns	.14	.21	ns	.09	.13	**	.11	.15	ns
11	.01	.03	**	.02	.03	ns	.02	.03	ns	.04	.04	ns	.01	.02	ns	.03	.04	ns
12	.09	.12	*	.72	.88	ns	.10	.13	*	.84	1.65	ns	.09	.11	ns	.74	1.09	**
13	.00	.03	ns	1.85	2.15	ns	.02	.05	ns	2.21	1.57	ns	.01	.03	ns	2.12	2.13	ns
14	2.44	1.43	**	2.52	2.66	ns	1.31	1.43	**	2.71	2.36	ns	2.16	1.14	*	2.85	2.97	ns
15	.05	.05	ns	1.10	1.32	ns	.09	.14	ns	1.40	0.99	ns	.03	.06	ns	1.16	1.16	ns
16	27.59	24.90	*	21.12	20.05	ns	27.16	24.76	*	22.18	17.30	*	25.75	24.19	ns	21.56	18.66	ns
16-1	4.32	4.76	ns	1.25	0.90	ns	4.65	4.32	ns	1.16	1.03	ns	4.56	4.10	ns	1.12	0.90	ns
17	.00	.00	ns	.32	.35	ns	.00	.00	ns	.39	.23	ns	.00	.00	ns	.33	.29	ns
18	9.63	9.97	ns	7.81	6.98	ns	9.61	16.56	*	7.94	9.92	ns	10.19	17.08	*	7.76	8.01	ns
18-1	47.95	50.07	ns	13.35	10.54	ns	48.72	45.08	ns	12.82	13.51	ns	49.51	45.63	ns	12.25	11.63	ns
18-2	4.61	5.24	ns	35.02	38.79	ns	4.72	4.23	ns	35.26	36.01	ns	4.47	4.24	ns	35.61	39.84	ns
18-3	2.35	2.47	ns	1.03	1.07	ns	2.62	2.30	ns	1.33	1.08	ns	2.33	2.46	ns	1.78	1.08	ns
20-4	0.88	0.79	ns	13.49	14.15	ns	0.86	0.84	ns	11.59	14.11	ns	0.79	0.80	ns	12.59	12.04	ns
18-1/18	4.98	5.20		1.71	1.51		5.07	2.72		1.61	1.36		4.86	2.67		1.58	1.45	

[a] Over-all sexes and weights, the fatty acids shown above were significantly ($P < .05$) different between fractions.
[b] Fraction 1 - chloroform extract (neutral lipid)
[c] Fraction 2 - methanol extract (phospholipid)
[d] Light weight group
[e] Heavy weight group
* $P < .05$
** $P < .01$

Table 2. Effect of sex on fatty acid composition of neutral lipid and phospholipid fractions[a,b]

Fatty acid No.	Neutral Lipid						Sex & wt. effect
	Light Weight			Heavy Weight			
	Boars	Barrows	Gilts	Boars	Barrows	Gilts	
10	0.08^{w}	0.09	0.12^{w}	0.13	0.13	0.13	ns
11	0.01^{w}	0.01	0.02^{w}	0.03	0.02	0.03	ns
12	0.09	0.09	0.10	0.12	0.11	0.13	ns
13	0.00	0.01	0.02	0.03	0.03	0.05	ns
14	2.44^{y}	2.16^{z}	1.30^{yz}	1.43	1.14	1.43	**
15	0.05	0.03	0.09	0.05	0.06	0.14	ns
16	27.59	25.75	27.16	24.90	24.19	24.76	ns
16-1	4.32	4.56	4.65	4.76^{w}	4.10^{w}	4.32	ns
17	0.00	0.00	0.00	0.00	0.00	0.00	ns
18	9.63	10.19	9.61	9.97^{wx}	17.08^{w}	16.56^{x}	*
18-1	47.95	49.51	48.72	50.07^{wx}	45.63^{w}	45.08^{x}	*
18-2	4.61	4.47	4.72	5.24	4.24	4.23	ns
18-3	2.35	2.33	2.62	2.47	2.46	2.30	ns
20-4	0.88	0.79	0.86	0.79	0.80	0.84	ns

[a]No difference for sex/light weight/phospholipid and sex/heavy weight/phospholipid.

[b]Means with same superscript are significantly different
w or x = $P < .05$
y or z = $P < .01$

Table 3. Sex[a] and weight analysis for longissimus dorsi

Intramuscular lipid content

Weight group	Boars mean %	Std. dev.	Gilts mean %	Std. dev.	Barrows mean %	Std. dev.
Light weight (49.6 kg)	2.81	0.43	3.10	1.02	3.18	0.75
Heavy weight (94.1 kg)	3.86	0.48	5.55	2.44	5.56	2.01
Weight effect	$P < .01$		$P < .10$		$P < .05$	

[a]Sex means were not significantly different from each other.