TRAMUSCULAR FAT CONTENT AND LIPID IN MUSCLE FIBRES OF PIGS FED HIGH AND LOW PROTEIN DIET AND RELATION TO MEAT QUALITY.

ESSEN-GUSTAVSSON[1], A. KARLSSON[2] , K. LUNDSTRÖM[3] and A-C. ENFÄLT[2]

Dept. of Medicine and Surgery[1], Dept.of Food Science[2], Dept. of Animal Breeding and Genetics[3]. Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

SUMMARY

Samples were taken from M. longissimus dorsi (LD) and M. biceps femoris (BF) immediately at exsanguination in a group of slaughter pigs of Halothane-gene-free Swedish Yorkshire (entire males and gilts) fed a high (HP-pigs) or a low (LP-pigs) protein diet. Triglyceride content (TG) was analysed after the samples had been freeze-dried and dissected free from blood, connective tissue and fat. Histochemical stainings were performed, on muscle samples from LD, to identify type I, IIA, IIB and IIC fibres and to evaluate lipid content and oxidative capacity within the fibre types. Intramuscular fat content (IMF), meat colour and drip loss were measured in both muscles. Shear force values (SF) were measured on cooked meat from LD.

IMF values were higher in both LD (2.3%) and BF (2.0%) of LP-pigs compared to LD (1.5%) and BF (1.4%) of HP-pigs. TG content did not differ between diets in BF but was higher in LD of LP-pigs compared to HP-pigs. In LD, but not in BF, a correlation was seen between IMF and TG content in the muscle. Fibre type composition did not differ between HP and LP-pigs. BF had a higher percentage of type I and IIA fibres and a lower percentage of type IIB fibres compared with LD. TG content in muscle showed no relationship to fibre type composition or staining intensity for lipids. High staining intensity for lipid in LD was mainly seen in type I fibres. Meat quality parameters did not differ between groups except SF -values which were higher in HP (4.7) than in LP-pigs (4.0). SF-values correlated negatively to TG (r= - 0.62) and IMF (r= - 0.75) content in LD. TG content was negatively correlated to mean fibre areas (r= - 0.70).

The results of this study show that neutral lipids are mainly stored in type I fibres and variations seen in IMF is to a minor extent related to differences in TG content in the muscle fibres. The results indicate that IMF and TG content in muscle fibres influence shear force values.

INTRODUCTION

Intramuscular fat (IMF) may influence meat quality and sensory properties (for ref. SCHWÖRER et al 1989). IMF in a muscle represents the lipid that is stored both intracellular and intercellular and between the fascicles. Skeletal muscle is composed of fibres having different contractile and metabolic characteristics. Lipid stored as droplets within the fibres may be used as a substrate in connection with muscle contraction. Intracellular lipid is predominantly deposited in slow-contracting type I fibres which have a higher oxidative capacity than fast-contracting type IIA and IIB fibres. The proportion of fibretypes having different metabolic characteristics vary among animals and among muscles depending on function. Data presented in the literature on IMF in pig muscles give an overall picture of the total lipid stores in a piece of muscle. The purpose of this study was therefore to use both histochemical and biochemical techniques to evaluate in more detail the lipid stored in muscle and within different fibretypes and to distinguish between intra- and extracellular lipidstores.

MATERIAL AND METHODS

Halothane-gene-free Swedish Yorkshire pigs (entire males and gilts) from a selection experiment were used in this study (STERN et al 1990). Twenty pigs (HP-pigs) were fed a high protein diet (18.5% crude protein, 0.96% lysine) and twenty pigs (LP-pigs) a low protein diet (13.1% crude protein, 0.54% lysine). The metabolized energy content was 11.9 MJ/kg in both diets. The pigs were slaughtered the week they had reached 103 kg. The pigs were transported 5 km from the research station to the slaughterhouse and kept for 2 hours in lairage before they were electrically stunned with low voltage on the floor. Immediately at exsanguination a muscle sample was taken from M.longissimus dorsi (LD) and from M.biceps femoris (BF). The piece of muscle sample taken for histochemical analyses was rolled in talcum powder before being frozen in liquid nitrogen. The sample taken for biochemical analyses was immediately frozen in liquid nitrogen. All samples were stored at - 80^{o} until analysed.

Histochemical analyses. Serial cross-sections were cut in a cryostat and stained for myofibrillar ATP-ase after both acid and alcaline preincubation in order to identify type I,IIA,IIB and IIC fibres (BROOKE AND KAISER 1970). Sections were also stained for NADH-tetrazolium reductase (NADH-TR) to evaluate oxidative capacity and with Sudan black B and Oil red O to evaluate lipid content (DUBOWITZ 1985). The percentage of fibretypes, fibre areas and the relative area of fibre types were evaluated with a computerized image analysis system (BIO-RAD, Scan Beam, Hadsund, DK).

Biochemical analyses. Muscle samples were freeze-dried and dissected free from blood, connective tissue and lipid droplets. As pure muscle as pos
was weighed (1-2 mg). Triglycerides were analysed by extraction of neutral fats from the muscle sample with a Folch extract (methanol/chlorof
The chloroform phase was retained and after evaporation hydrolysed and the glycerol content was determined (CHERNICK 1969)

Meat quality analyses. Technological meat quality was measured 24 hours post mortem. Meat colour was measured as surface reflectance (
Diffusion Systems Ltd, London, England) and drip loss was measured according to HONIKEL (1987). Intramuscular fat content (IMF) was anal
using the Soxtec System H+ equipment (Tecator AB, Höganäs, Sweden). Shear force values (SF) were measured on meat aged for two days be
freezing, using the Warner-Bratzler apparatus according to the procedure of LUNDSTRÖM et al (1987).

RESULTS AND DISCUSSION

IMF values were higher in both LD (2.3%) and BF (2.0%) of LP-pigs compared to LD (1.5%) and BF (1.4%) of HP-pigs. TG-content did not
between diets in BF but was higher in LD of LP-pigs compared to HP-pigs (table 1). When considering HP and LP-pigs as one group, a relationship
seen between IMF and TG content in LD (r= 0.55) but not in BF. Fibre type composition did not differ between HP and LP-pigs but a difference
seen between muscles (table 1). One could expect a higher TG content in BF as this muscle had more type I and IIA fibres and less type IIB fibres
LD (table 1). The results show however, no relationship between fibre type composition and lipidcontent in muscle measured either as IMF or
content. TG content was almost double in LP-pigs compared to HP-pigs in LD which contained less than 10 % type I fibres. A variation in TG co
was also seen in different breeds of pigs with similar fibre type composition in LD (ESSEN-GUSTAVSSON AND FJELKNER-MODIG 1985). A s
on crossbred pigs have recently shown that intramuscular lipid content and TG-content vary little with metabolic type of muscle (LESEIGNEU
MEYNIER AND GANDEMER 1991). That study also showed that a piece of LD muscle contained 1.0 % triglycerides and 0.5 % phospholipids
total lipid content was 1.5 %. The data were obtained from a piece of muscle which likely contained both intercellular and intracellular lipid sto
can be calculated from the data in the present study, assuming a molecular weight of around 800 for triglycerides, that TG content within the m
fibres of LD only reflects a small fraction (0.38± 0.26 %) of total lipids. Muscle samples with high TG content (20-40 mmol/kg d.w.) indicate that
0.8% of neutral lipids can be stored in the muscle fibres.
The histochemical stainings were performed on LD samples from 14 pigs with a large variation in IMF values between individuals (range 0.77-4.93
High staining intensity for lipid as evaluated from the Oil red O stain was seen in all type I fibres, in 26% of type IIA fibres and only in 1 % of type
fibres (table 2). With the Sudan black B stain all type I fibres showed a high staining intensity and this was also seen in about 50% of type IIA fib
and 3% of type IIB fibres (table 2). Fibres that were unstained in Oil red O showed medium intensity for Sudan black B in 36 % type IIA fibres and
15 % type IIB fibres. It is said that the Oil red O stain evaluates neutral lipids whereas the Sudan black B stain evaluates all types of lipids inclu
phospholipids. The NADH-TR stain which indicates oxidative capacity of a fibre showed an almost similar pattern for staining intensity as the Su
black B stain. The area of fibres with high staining intensity for Oil red O, Sudan black B and NADH-TR was calculated and compared to the t
area. These calculations showed that LD is a muscle with limited oxidative capacity and lipid content since only about 7 % of the total area to
red O and about 25 % for Sudan black B and NADH-TR showed high staining intensity. The evaluation of lipidcontent from the histochemical st
showed no relationship to the quantitative analyses of TG content in the muscle. Histochemical stains are only semi-quantitative and lipid co
within fibres classified as having a high staining intensity could therefore vary. Of note was the negative correlation seen between TG content
mean fibre area (r= - 0.70) and mean area of type IIB fibres (r= - 0.74). The total cross-sectional area consists of around 90 % type IIB fibres w
explains why IIB fibre areas show a close correlation (r=0.98) to mean areas. If mean area is lower in a muscle one would expect a hig
capillarisation of the muscle (cap/mm^2). A high capillarisation in the muscle would facilitate transportation and deposition of lipid in a fibre. In pig
different halothane genotypes, a negative correlation is seen in LD between mean fibre area and capillarisation (ESSEN-GUSTAVSSON et al 198
Meat quality parameters did not differ between the HP and LP-pigs in this study except for shear force values which were analysed on LD (table
SF-values correlated negatively to TG content (r = - 0.62) and IMF values (r = - 0.75) but no relationship was seen to any of the parameters
evaluated from the histochemical stains. It has been shown that the amount of fat evaluated from histological observations on meat from
correlates positively to tenderness measured by a taste-panel and negatively to shear force values (CARPENTER et al 1963). Several studies
different breeds of pigs have shown that a high oxidative capacity, a high IMF and a high TG content in muscle have a positive influence on m
quality and sensory properties (ESSEN-GUSTAVSSON AND FJELKNER-MODIG 1985, REDE et al 1986,TOURAILLE et al 1989, SUZUKI et al 19

CONCLUSION.

This study shows that neutral lipids are mainly stored in type I fibres and that the variations seen in IMF is to a minor extent related to differences
TG content within muscle fibres. The results indicate that high TG content in muscle fibres may be related to low shear force values.

Table 1. Mean±s.d. of intramuscular fat content (IMF), triglyceride content (TG), fibretype composition, meat colour (EEL), drip loss and shear force values analysed on muscles from twenty pigs fed a high protein diet (HP-pigs) and twenty pigs fed a low protein (LP-pigs) diet.

	M. BICEPS FEMORIS		M. LONGISSIMUS DORSI	
	HP-pigs	LP-pigs	HP-pigs	LP-pigs
IMF (%)	1.4±0.4	2.0±0.5	1.5±0.4	2.3±0.9***
TG (mmol/kg d.w.)	15±10	17±9	12±5	22±11***
FIBRE TYPE (%)				
I	21±7	22±5	7±4	8±4
IIA	8±4	11±4	7±3	8±3
IIB	70±9	66±8	84±5	82±6
IIC	1±1	1±1	2±1	2±2
DRIP LOSS (%)	2.4±1.2	2.4±1.4	4.0±1.5	4.1±1.6
EEL VALUE	16.2±2.5	15.7±2.6	20.5±4.2	19.6±3.0
SHEAR FORCE (kg/cm2)	-	-	4.7±0.7	4.1±0.8*

Level of significance within muscle: *=p≤0.05, ***=p≤0.001

Table 2. Mean±s.d. for staining intensity in different fibretypes evaluated as high, medium and low with an image analysis systyem. Samples (M. Longissimus dorsi) from fourteen pigs were stained for Oil red O, Sudan black B and NADH-tetrazolium reductase (NADH-TR).

Fibretype (%)	Oil red O	Sudan black B	NADH-TR
I			
High	100±0	100±0	100±0
IIA			
High	26±35	55±33	51±33
Medium	0	36±35	44±30
Low	74±35	9±11	5±7
IIB			
High	1±1	3±4	4±5
Medium	0	15±6	17±7
Low	99±2	82±7	79±6

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