FATTY ACIDS,CHOLESTEROL AND ITS DERIVATIVES IN DIFFERENT CATTLE TISSUE LIPIDS

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INTRODUCTION

Fatty acid composition of meat lipids in relation to race, sex, age, feeding, anatomical location and other factors has been comprehensively studied. However there are no precise data on fatty acid composition as well as on lipid ones of certain by-products which application has been growing more extensively for various purposes.

Veal as light colored meat with low fat content is popular in many countries. In fact the production parameters for veal have changed significantly within the past few years, slaughter weights of cattle increasing from less than 7o kg to up the 4oo kg liveweight.

Several investigations have already dealt with the fatty acid composition of cattle tissues as related to breed and sex (1,2,3) to the point of weaning (4) or to increasing amounts of different fatty acids in the diet (5).

The recognition of lipid types in a mixture and their quantitative determination is achieved mainly by chromatographic separation.
The most widely used procedures are based on adsorption chromatography with silica or acid-washed florisil. Neutral lipids are eluted from a column with chloroform, glycolipids with acetone and phospholipids with methanol (6,7).

Lipids studies are important not only from the nutrition point but also they are of interesent in studing changes of individual raw materials and foods in the course frozen storage.

The purpose of this work was to examine the extent of differences in total lipid content and fatty acid composition and content of Cholesterol and its derivatives of cattle tissues:muscle, liver, fatty tissue, brain and spinal cord.

Present study gives also data on fatty acids composition of total lipids as well as of phospholipids and glucolipids.

MATERIALS AND METHODS

The muscle tissue, livers, fatty tissues, brains and spinal cord of five Simmental cattle weighting abut 4oo kg and 18 months old were taken from the slaughter line.
All the tissues were sealed in PE bags and held at -3o°C until further processing.

The total lipids were extracted by procedure according to Folch et al (8) at 15°C. The lipid fractions were fractionated on a Silica Gel 6o (7o-23o mesh) column to neutral lipids, glucolipids and phospholipids according to the procedure described by Johnston et al (9). Mass content of fractions were determined gravimetrically after the evaporation of solvent in a nitrogen gas stream and expressed as percentage of total lipids

The methyl esters of fatty acids of total lipids, phospholipids and glucolipids were prepared with diazomethane(1o). The identification and the quantitative analysis were performed on a Varian 34oo capillary gas chromatograph (SE 54 fused silica capillary stationary phase on deactivated siloxane,column lenght 25 m, inner diameter o,25 mm) with a FID detector. Nitrogen was used as a carrier gas with flow rate 1.18 ml/min. Injector and detector temperatures were 25o° and 3oo° respectively. The analysis were performed at heating rate of 4°/min (13o°-29o°C).

Methyl esters of C_{1o} to C_{2o}, C_{22} and C_{24} saturated and unsatarated fatty acids

were used as identification standards.

The compounds were identified by comparing them with reference substances and mass spectra obtained by comparison with library mass spectra from Göttingen University.

The percentage of total surface area in quantitative analysis, obtained by Spectra Physics System I Computing Integrator, were converted to percent mass by comparing with mixtures of acid methyl esters of known composition.

RESULTS AND DISCUSSION

The results shown in Table 1.indicate high differences not anly in quantities of total lipids but also in relative proportion of certain lipid fractions in muscle tissues, liver, fatty tissues, brain and spinal cord.

Table 1. The mean composition of lipids isolated from tested tissues

Tissue	Total lipids*	Neutral lipids**	Gluco-lipids**	Phospho-lipids**
Muscle tissue	2.92	75.1o	2.o8	22.82
Liver	3.84	24.81	3.12	72.o2
Fatty tissue	89.95	96.56	o,41	3.o3
Brain	9.62	34.51	7.o8	58.41
Spinal cord	2o.98	24.85	3.78	71.36

* on the whole tissue basis ** on the total lipid basis

There are particularly great differences in proportions of glucolipids and phospholipids in total lipids among individual tissues.

The highest quantities of phospholipids are found in liver (72.o2 %) and of glucolipids in brain (7.o8 %).

Therefore, besides the comparative study of the fatty acid composition of total lipids, the work was oriented to the comparative fatty acid composition study of phospholipids and glucolipids.

Table 2. shows relative proportions of saturated (S), monounsaturated (M) and polyunsaturated (PUFA) fatty acids in total lipid extracts from tested tissues, and Table 3. shows carbonic acids distribution of total lipids in the same cattle tissues.

Data on relative participations of saturated, monounsaturated and polyunsaturated fatty acids in phospholipids of tested tissues (Table 4) show high quantities of polyunsaturated fatty acids in brain (26.93%) and particulary in spinal cord (54.85%) which is fifteen to twnty times higher than in phospholipids of muscle tissue and fatty tissue. So brain PUFA/S ratio is o.6 and for spinal cord it is 3.32 (Table 4).

In the phospholipid fraction of all tested tissues the linolenic acid ($C_{18}^{3=}$) is present in the highes quantities in spinal cord (37.7%) wheres in fatty tissue (1.16%) and in liver (2.78 %).

On the other hand linoleic acid ($C_{18}^{2=}$) is present in the highes quantities in brain (23.51%) wheres in other tissues is much less.

In monounsaturated acids there is also a high participation of oleic acid being 37.9 % in muscle tissue, 15.69 % in spinal cord, 12,39 % in fatty tissue, 8.74 % in brain and 2.39 % in liver.

Stearic acid (C_{18}) is present in following quantities in phospholipid fractions: 5o.72 % in liver, 21.14% in brain,

15.6 % in muscle tissue, 1,39% in fatty tissue and 1.33 % in spinal cord.

Phospholipid fraction is particulary characterised by high quantities of long-chain polyunsaturated fatty acids as well as monounsaturated ones especially in spinal cord and in brain.

Regarding relative proportions of saturated, monounsaturated and polyunsaturated fatty acids in glucolipids from those five investigeted tissues (Table 5) it is remarkable that muscle tissue are the richest in saturated acids (29.98%) while unsaturated fatty acids are most concentreted in fatty tissue.

Table 2. Relative proportions of saturated (S), monounsaturated (M) and polyunsaturated (PUFA) fatty acids in total lipid extracts from tested tissues

Tissue	S	M	PUFA	PUFA/S
Muscle tissue	45.06	7.56	31.06	0.68
Liver	43.61	6.85	27.96	0.64
Fatty tissue	38.6	44.2	2.25	0.05
Brain	17.23	4.54	15.84	0.92
Spinal cord	30.17	17.57	39.67	1.31

Table 3. Carbonic acids distribution of total lipids in different cattle tissues represented as percentage of total area of corresponding GC-chromatogram

No of peak in corresponding Figs (1-5)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Tissues	C_{10}	C_{12}	$C^{1=}_{14}$	C^{o}_{14}	$C^{1=}_{15}$	C^{o}_{15}	$C^{1=}_{16}$	C^{o}_{16}	$C^{1=}_{17}$	C^{o}_{17}	$C^{3=}_{18}$	$C^{2=}_{18}$	$C^{1=}_{18}$	C^{o}_{18}	$C^{1=}_{19}$	C^{o}_{19}	$C^{5=}_{20}$	$C^{4=}_{20}$	$C^{3=}_{20}$	$C^{2=}_{20}$	$C^{1=}_{20}$	C^{o}_{20}	$C^{3=}_{22}$	$C^{2=}_{22}$	$C^{1=}_{22}$	C^{o}_{22}	$C^{1=}_{24}$	C^{o}_{24}	$C^{1=}_{25}$	C_{25}
Muscle tissue	0.1	tr	0.24	1.46		0.33	0.16	24.23	0.7	1.02		30.0	4.0	17.57		0.15			0.25	0.53	0.56	0.2	0.15	0.13	tr	tr		tr	tr	
Liver		0.1	tr	0.38	0.09	0.18	0.50	10.39	0.56	0.80	8.77	11.91	2.24	30.85	0.5	0.6	--	3.94	1.05	0.49	0.46	0.16	0.8	1.00	2.5	0.15		tr	tr	
Fatty tissue			0.8	2.07		0.29	2.87	23.26	0.57	0.86			36.26	10.78		0.28					2.41	0.68		2.25	1.29	0.38		tr		
Brain	-	-		0.20	0.09	0.15	0.26	6.67	0.07	0.25		6.78	2.46	9.05	0.1	0.07		0.17	0.21	1.07	0.63	0.18	0.35	0.10	0.23	0.11	0.30	0.25	0.4	0.3
Spinal cord	0.17	0.29	0.79	2.07	tr	0.3	2.87	23.26	tr	0.76		36.26	10.78	1.04		0.7		0.5	0.5	2.41	0.68	0.2			2.25	1.28	0.2	0.1	tr	0.1

Table 4. Relative proportions of saturated (S), monounsaturated (M)and polyunsaturated (PUFA) fatty acids in phospholipids from tested tissues

Tissue	S	M	PUFA	PUFA/S
Muscle tissue	38.7	41.72	1.68	0.043
Liver	64.32	3.03	21.0	0.33
Fatty tissue	8.09	12.39	1.98	0.244
Brain	44.67	11.71	26.93	0.6
Spinal cord	16.51	19.02	54.85	3.32

Table 5. Relative proportions of saturated (S) monounsaturated (M) and polyunsaturated (PUFA) fatty acids in glucolipids from tested tissues

Tissue	S	M	PUFA	PUFA/S
Muscle tissue	29.98	1.52	5.97	o.2
Liver	2o.32	2.52	6.2	o.3
Fatty tissue	21.14	17.58	4.o3	o.19
Brain	23.89	4.21	27.48	1.15
Spinal cord	9.31	12.61	41.61	4.46

Fatty acid composition of glucolipids is much less complex, slightly more complex are spinal cord and brain glucolipids.Especialy in brain and in spinal cord there are long-chain polyunsaturated and monounsaturated acids. There is a high quantities of unsaturated acids with 2o C-atoms ($C_{2o}^{4=}$) in brain glucolipids especialy (arachi-donic acid).

The total lipids were extracted from particular tissue according to procedure by Folch (8). Diethylether extract after hydrolysis contained the free fatty acids and also cholesterol and its derivetives. In Figures 1-5 gas chromatograms of fatty acid methyl esters are presented, together with steroid components in the extract.

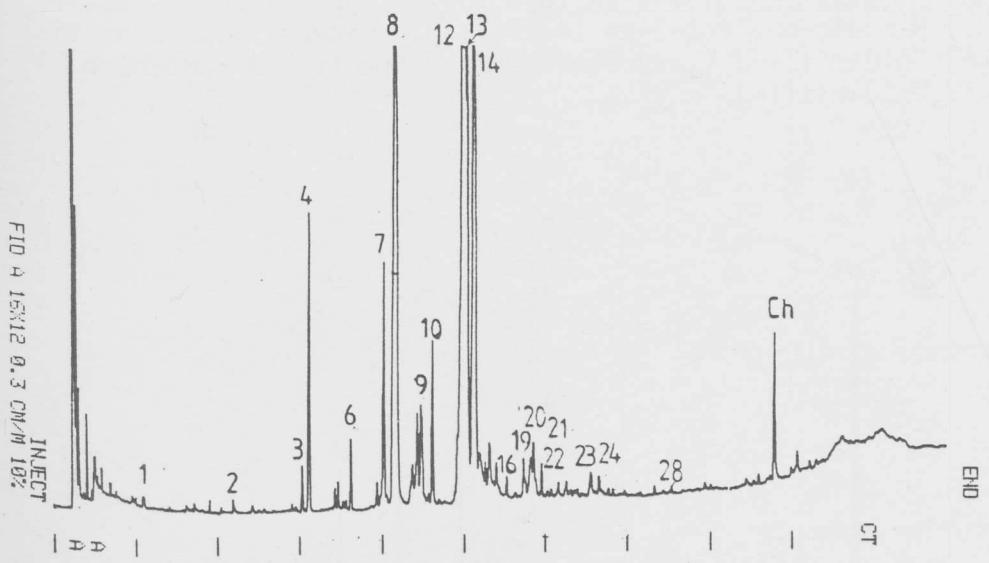

Fig. 1. - GC chromatogram of acid methyl-esters cattle muscle tissue.Ch (cholesterol)

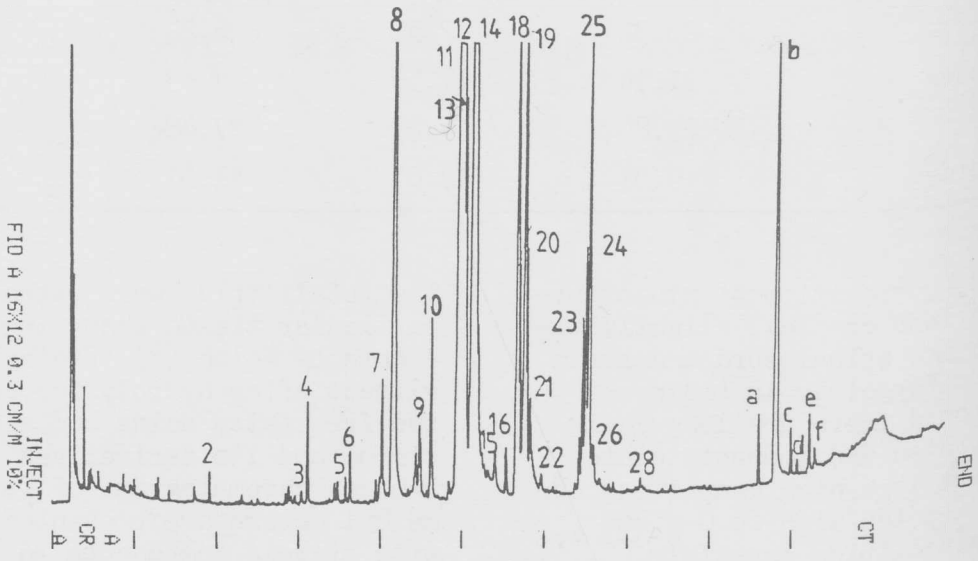

Fig. 2.-GC chromatogram of acid methyl esters cattle liver tissue
a) 3-methoxy-choles-5-en (o,26%); b) cholesterol (4.18%);
c) 3-aceta-cholest-5-en (o.18%); d) Unidentified (steroidal type);
e) Unidentified (very similar mass spectra as component d)
f) Unidentified

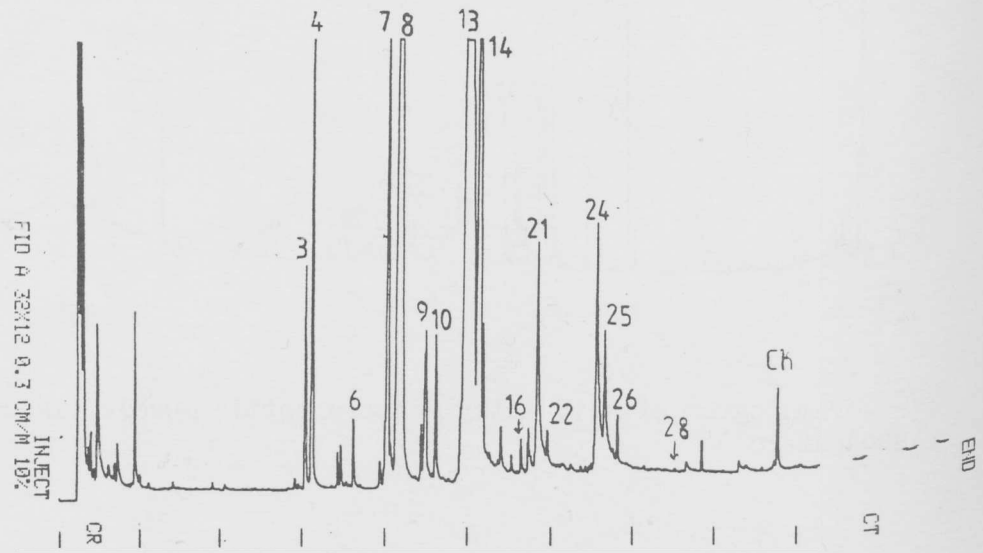

Fig. 3.- GC chromatogram of acid methyl esters cattle fatty tissue. Ch (cholesterol)

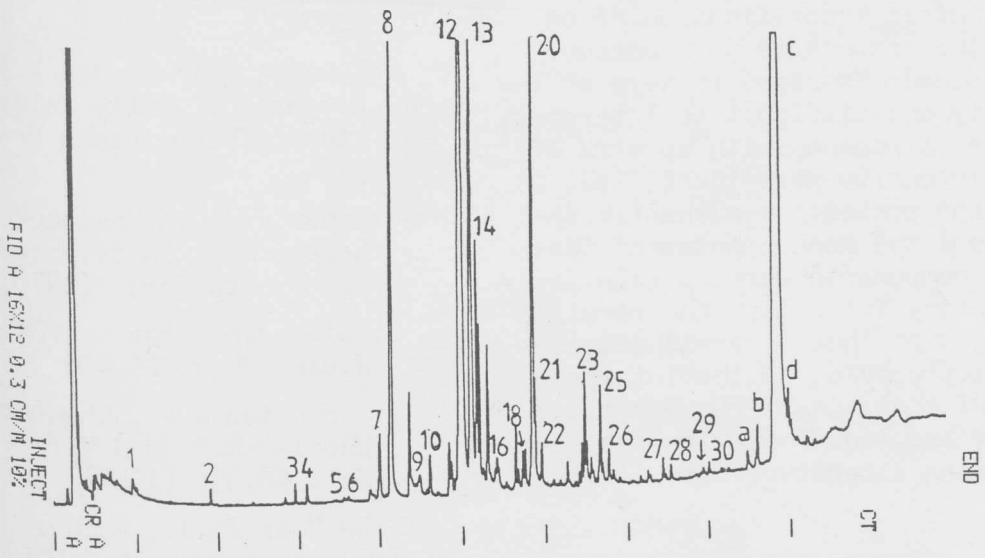

Fig. 4. GC chromatogram of acid methyl esters cattle spinal cord tissue
a) Unidentified (steroidal type); b) cholesta-3,5-diene ($C_{27}H_{44}$);
c) Cholesterol; d) 3β-hydroxy-5α-cholest-7-en

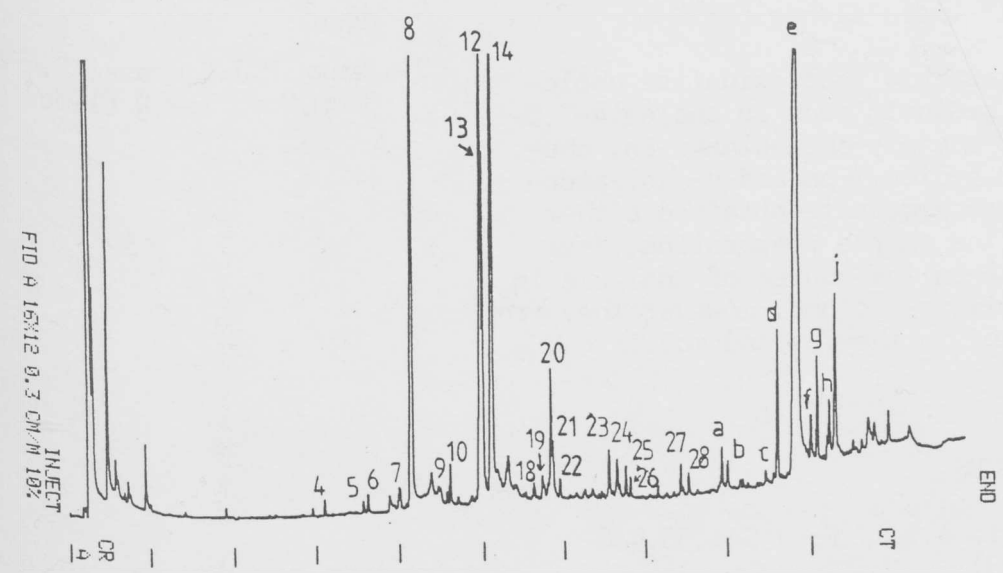

Fig.5. GC chromatogram of acid methyl esters cattle brain tissue
a) cholesta-4,6,8(14)-trien; b) Unidentified; c) Cholesta-3,5-dien;
d) 3-methoxy-cholest-5-en (1.2%); e) cholesterol; f) 3-acetoxy-cholestan-6-on (o.9%); g) cholesta-3,5-dien-7-on (1.4%); h) Unidentified (1.o9%); j) 4-cholesten-3-on (2.74%)

The percentages and corbon acid distribution of total lipids in tested tissues are given in Table 3, under the corresponding peak numbers. Together with cholesterol other steroid components were detected, mass spectrometric identification of certain microcomponents was tedious. The obtained mass spectra were of low intensity and difficult to interpret, only the components with spectra of higher intensity were identified. As we did not posses the authentic samples, and the mass spectra of identified components were of relatively satisfactory intensity, the possible structures of those steroid components were proposed, as the interpretation of their mass fragmentations is clear and identical with the corresponding literature data (11,12, 13,14).

In this manner, besides cholesterol which is present in all investigated tissues, the following steroid substances were also identified: 3-methoxy-cholest-5-en and 3-acetacholest-5-en in liver tissue, cholesta-3,5-dien and 3β-hydroxy-5α-cholest-7-en in spinal cord tissue, cholesta-4,6,8 (14)-trien, cholesta-3,5-dien, 3-acetoxy-cholestan-6-on, cholesta-3,5-dien-7-on and 4-cholesten--3-on in brain tissue.

It is possible that oxidative cholesterol products such as cholesta-3,5-dien, 3-acetoxy-cholestan-6-on, cholesta-3,5-dien-7-on and 4-cholesten--3-on are artefacts obtained either during the sample preparation, less possible in the course of analysis in the capillary column. However they were not found in the fat and muscle cattle tissues.

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