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# Atherogenic and thrombogenic index, acidic composition of lipid fraction of adipose subcutaneous tissue of female ancient autochthonous genetic type (AAGT) *Casertana*. Preliminary results<sup>1</sup>

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**SUMMARY** – The research was performed on samples of adipose subcutaneous and perirenal tissues of 5 *Casertana* sows, reared in multiple boxes at the ConSDABI experimental farm and slaughtered at ~160 kg of live weight. *Casertana* is an ancient autochthonous genetic type (AAGT), present nowadays mainly in Campania (Italy), where there is in progress an extensive programme recovery promoted by ConSDABI. The aim of this study was to characterize the lipid component of adipose tissue evaluated for "chemical-nutritional" and "chemical-extranutritional" quality. The results showed that, in all samples examined, the most abundant fatty acids were the C18:1 and C18:2; among the saturated fatty acids, the highest values being observed for C16:0. In the case of adipose subcutaneous sites the UFA/SFA ratio is about 2.30, while the perirenal fat shows a UFA/SFA ratio of about 1.60. These results highlighted that the subcutaneous adipose tissue of *Casertana* AAGT is a source of fatty acids with functional features to human health and therefore can be conveniently inserted in a correct diet.

**Key words:** *Casertana* pig, ancient autochthonous genetic type, atherogenic index, thrombogenic index.

**RESUME** – "Index athérogénique et thrombogénique, composition acide de la fraction lipidique du tissu adipeux sous-cutané de femelles de l'ancien type génétique autochtone (TGAA) *Casertana*. Résultats préliminaires". L'étude a été conduite sur des échantillons de tissu adipeux (sous-cutané et périrénal) de porcs femelles TGAA *Casertana*, élevées dans des box multiples près de la structure expérimentale du ConSDABI et abattues à un poids moyen proche de 160 kg. La *Casertana* est un type génétique autochtone ancien (TGAA) présent, principalement en Campanie (Italie) où est menée une vaste récupération promue par le ConSDABI. L'objectif de ce travail est de caractériser la composante lipidique du tissu adipeux examiné pour évaluer la qualité "chimique-nutritionnelle" et "chimique-extranutritionnelle". Les résultats ont mis en évidence : les acides gras les plus abondants sont C18:1 ; C18:2 et C16:0. Dans le tissu adipeux sous-cutané le rapport UFA/SFA est proche de 2,30 ; dans le tissu périrénal, il est proche de 1,60. Le tissu adipeux sous-cutané du TGAA *Casertana*, donc, peut être convenablement intégré dans un régime alimentaire correct.

**Mots-clés :** *Casertana*, type génétique autochtone ancien, index athérogénique, index thrombogénique.

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## Introduction

The increasingly trend of human nutritional studies towards the reduction of fats in human diet has determined, in recent years, a considerable decrease of the consumption of foods of animal origin, above all pork meat.

The pig is a monogastric animal that, like ruminants, normally eats vegetable feeds. However,

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unlike ruminants, it cannot biohydrogenate vegetable fats; this causes a higher presence of unsaturated fatty acid in the intramuscular and storage fat in comparison with the other animals for slaughtering. Moreover, unlike vegetable fat and fat from other mammals, the triglycerides of pork fat have unsaturated fatty acid in sn position (stereochemical numbering) 1 and 3 and the saturated fatty acid in position 2 (di Luccia *et al.*, 2003). These traits make meat of this species very interesting, since the unsaturated acids are immediately available; indeed, the human digestive lipase hydrolyzes the triglycerides firstly in position sn1 and sn3 and, secondly, by means of isomerase, which shifts the fatty acid from position 2 to position 1 or 3, hydrolyzing the triglyceride fully. A major availability of saturated fatty acids can affect positively human feeding, implying a decrease of saturated fatty acid (SFA), improving the UFA (unsaturated fatty acids)/SFA ratio, to which an increasingly important role is ascribed in prevention and/or reduction of cancer, atherosclerosis and obesity (Nielsen *et al.*, 1995; Williams, 2000).

The typifying of *lipidome* (set of lipids and their interactions in an organism), is important in order to better understand the qualitative production potentiality of a genetic resource. In the last years the evolution of the studies on qualitative traits of pork meat [i.e. the meat from pig ancient autochthonous genetic type (AAGT) *Casertana*], is allowing to highlight some health aspects of the same, contributing to its reassessment, above all in relation to the high nutritional power of some UFA [monounsaturated (MUFA) and polyunsaturated (PUFA)], present in adipose tissue.

The present study, through the determination of fatty acid composition in adipose and perirenal tissues, would contribute to the recovery and valorisation of *Casertana* AAGT, an ancient pig already described in I century BC by latin writer Lucio Giunio Moderato Columella, in his *De re rustica*.

## Materials and methods

### Sampling

The sampling concerned: (i) subcutaneous adipose tissue in thoracic site (SPT) at V-VII vertebra, distinctly for outer layer (SE) and inner layer (SI), and in lumbar site (SPL) at IV-VI vertebra, distinctly for SE and SI; and (ii) perirenal adipose tissue. The samples were stored at -30°C up to the moment of their use for quali-quantitative analysis of acidic fraction performed according to the AOAC methods (1994).

### Total lipid fraction extraction

50 g of adipose tissue was used for extracting total lipids by methanol-chloroform (2:1 vol/vol), according to the method of Folch *et al.* (1957).

### Triglycerides determination

The lipid samples (30 mg) were dissolved into 1 ml of hexane; 1 µl of this hexane solution was injected into a gas-chromatograph (Thermo Quest S.p.A., Milan) equipped with a fused silica capillary column RTX65TG (30 m x 0.25 mm i.d.; Restek Co., Bellafonte PA, USA). The temperature gradient used for the triglyceride separation was the following: from 340 to 360°C, 1°C/min; the injector and revealer temperature was 380°C; the moving phase flow (helium) was 1 ml/min with a split rate of 100:1. The quantitative values were calculated on chromatograms recorded by a Chrom Card for Window, previously identified and compared with a suitable external standard and with other chromatograms of known samples.

### Fatty acid determination

Methylic esters of fatty acids were prepared adding 1.25 ml of heptane to a drop of fat placed in a test tube with screw plug and subsequently adding 0.25 ml of methanolic KOH to the same test tube; the sample obtained was subjected to sonication for 6 min, at the end of which 1 µl of supernatant

was injected in a gas-chromatograph 5890 HP equipped with a fused silica capillary column fusa SP2380 30 m x 0.25 mm i.d. (Supelco, Inc., Bellefonte PA, USA); the used temperature gradient was the following: from 140 to 240°C, 1°C/min; the carrier gas flow, helium, was 1.50 ml/min. The chromatograms were recorded and pick areas were calculated by Agilent Chemstation; the identification was achieved through external standards (Larodan Lipids, ME 100). The obtained results were object of partial statistical elaboration.

## Results and discussion

The composition of depot fat of anatomical parts under examination is reported in Tables 1, 2 and 3. The determination of acidic composition of lipid fraction showed that the percentage incidence of fatty acids and the UFA/SFA ratio vary in relation to the adipose tissue under examination (subcutaneous or perirenal), as well as, as regards to subcutaneous adipose tissue, in relation to the thoracic (SPT) and lumbar (SPL) site of sampling and to the layer investigated (SE or SI). The comment of results has validity within the observation field.

Table 1. Subcutaneous adipose tissue (backfat). Mean value, standard deviation and variation coefficient (cv %) of percentage incidence of some fatty acids<sup>†</sup>, distinctly for sampling site and layer

Fatty acid	Sampling site							
	Thoracic (SPT)				Lumbar (SPL)			
	††SI		††SE		††SI		††SE	
	$\bar{x} \pm \sigma$	cv %	$\bar{x} \pm \sigma$	cv %	$\bar{x} \pm \sigma$	cv %	$\bar{x} \pm \sigma$	cv %
Myristic (C14:0)	1.15±0.29	25	1.03±0.10	10	1.06±0.07	7	1.10±0.15	14
Palmitic (C16:0)	19.94±1.78	9	17.88±1.18	7	18.64±1.02	5	18.35±1.90	10
Palmitoleic (C16:1)	0.47±0.10	21	0.43±0.04	9	0.45±0.06	13	0.43±0.08	19
Cis Palmitoleic (C16:1cis)	1.62±0.32	20	1.81±0.22	12	1.70±0.12	7	1.75±0.20	11
Heptadecanoic (C17:0)	0.28±0.05	18	0.21±0.02	10	0.22±0.03	14	0.21±0.04	19
Heptadecenoic (C17:1cis)	0.17±0.06	35	0.23±0.06	26	0.18±0.05	28	0.19±0.06	32
Stearic (C18:0)	9.61±1.50	16	8.60±0.97	11	8.91±0.81	9	8.55±0.73	9
Oleic (C18:1)	41.32±1.10	3	42.75±0.57	1	43.17±1.50	3	43.59±2.06	5
Linoleic (C18:2)	19.88±1.40	7	20.21±1.75	9	19.67±1.26	6	19.44±1.24	6
Linolenic (C18:3)	1.62±0.29	18	2.26±0.50	22	1.83±0.23	13	1.98±0.47	24
Eicosenoic (C20:1)	1.11±0.14	13	0.76±0.59	78	0.90±0.43	48	0.93±0.45	48
Beenic (C22:0)	0.15±0.07	47	0.23±0.10	43	0.19±0.03	16	0.17±0.08	47
Erucic (C22:1)	0.24±0.10	42	0.50±0.11	22	0.27±0.13	48	0.26±0.14	54

<sup>†</sup>The following fatty acids: Myristoleic (C14:1), Arachic (C20:0), Arachidonic (C20:4), Lignoceric (C24:0) and Selacoleic (C24:1), even though determined, were not reported because their average percentage incidence was lower than 0.05.

††SI = inner layer.

†††SE = outer layer.

The fatty acids mostly represented in adipose tissue under examination (subcutaneous and perirenal) were: palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2). Concerning these acids, the two types of adipose tissues were characterized by a different composition. Indeed, the subcutaneous adipose tissue presented a higher content of C18:1 and a lower content of C18:0 and C18:2 in comparison with the perirenal adipose tissue.

Table 1 shows: (i) oleic acid (C18:1) had a mean percentage value varying from a minimum of 41 in SI of SPT to a maximum of 44 in SE of SPL, the variation, expressed as variation coefficient (cv %), was between a minimum of 1 in SE of SPT and a maximum of 5 in SE of SPL; (ii) linoleic acid (C18:2) had a mean percentage value ranging from a minimum of 19 in SE of SPT to a maximum of 20 in SE of the same sampling site, the cv (%) was between a minimum of 6 in SI of both layers of SPL and a maximum of 9 in SE of SPT; (iii) palmitic acid (C16:0) had a mean percentage value varying from a minimum of 18 in SE of SPT to a maximum of 20 in SI of the same sampling site, cv (%) was between a minimum of 5 in SI of SPL and a maximum of 10 in SE of the same sampling site; and (iv) stearic acid has a mean percentage value ranging from a minimum of 8 in SE of SPL to a maximum of 10 in SI of SPT, the cv % was between a minimum of 9 in both layers of SPL and a maximum of 16 in SI of SPT.

Examining Table 2 it can be evidenced that the mean percentage values were: (i) oleic acid (C18:1) 29% (cv % = 7); (ii) linolenic acid (C18:2) 27% (cv % = 11); (iii) palmitic acid (C16:0) 22% (cv % = 5); and (iv) stearic acid (C18:0) 14% (cv % = 11).

Table 2. Perirenal adipose tissue. Mean value, standard deviation and variation coefficient (cv %) of percentage incidence of some fatty acids<sup>†</sup>

Fatty acid	$\bar{x} \pm \sigma$	cv %
Myristic (C14:0)	1.24±0.23	19
Palmitic (C16:0)	21.64±1.19	5
Palmitoleic (C16:1)	0.51±0.28	55
Cis Palmitoleic (C16:1cis)	0.87±0.19	22
Heptadecanoic (C17:0)	0.40±0.10	25
Heptadecenoic (C17:1cis)	0.14±0.03	21
Stearic (C18:0)	14.30±1.57	11
Oleic (C18:1)	29.44±2.15	7
Linoleic (C18:2)	26.98±3	11
Linolenic (C 18:3)	1.10±0.17	13
Eicosenoic (C 20:1)	1.36±0.17	15
Beenic (C 22:0)	0.23±0.10	43
Erucic (C 22:1)	0.20±0.13	65

<sup>†</sup>The following fatty acids: Myristoleic (C14:1), Arachic (C20:0), Arachidonic (C20:4), Lignoceric (C24:0) and Selacoleic (C24:1), even though determined, were not reported because their average percentage incidence was lower than 0.004.

Therefore, the mean value and the individual variability showed a certain determinism of AAGT. The oleic, linoleic, palmitic and stearic fatty acids, in both sampling sites (SPL and SPT), contribute to acidic fraction of lipidic mean composition for 90%, value equal to 92% in perirenal adipose tissue.

Moreover, meanly, in perirenal adipose tissue oleic and linoleic acids content was lower (56%) than that present in subcutaneous adipose tissue (63). The major presence of oleic acid in adipose tissues suggests a major activity of stearyl-CoA  $\Delta^9$  desaturase (Kouba *et al.*, 1997; Kouba and Mourot, 1998) that corresponds to a lower content of stearic acid in the same tissues. Comparing linoleic content in depot layers under examination, we deduce that it is present in similar content. This suggests that it is of exogenous source rather than derived from an enzymatic unsaturation.

Oleic and linoleic fatty acids are the most important unsaturated fatty acids; they contribute to enrich the aromatic component (Shródter *et al.*, 1986; Ramarathnan *et al.*, 1991; Elmore *et al.*, 1999) and present a high nutritional power for their protective role against cardiovascular diseases (Hornstra, 1999). Saturated fatty acid, in particular palmitic and stearic, contribute for about 10% in both sites. As concerns UFA/SFA ratio, it results equal to 1.60 in perirenal adipose tissue, with 38% of

saturated fatty acid, while in subcutaneous adipose tissue such ratio results equal to 2.30, with 31% of saturated fatty acid; moreover, independently from the sampling site, the outer layers present a major value of UFA/SFA ratio than inner ones. This acidic composition is in agreement with guidelines for a correct alimentation which suggest a decrease of saturated fatty acid intake. Indeed, in a correct dietary, according to the WHO rules (World Health Organization) for heart healthy, the recommended energy intake coming from saturated fatty acid should be major than 10% out of the total energy of dietary (FAO/WHO, 1998) with an UFA/SFA ratio higher than 2 (Capita and Alonso-Calleja, 2003).

However it must be highlighted that, even if saturated fatty acid *sensu lato* are involved in atherogenic and thrombogenic processes, not all of them express the same behaviour as regards the increase of serum cholesterol. Lauric (C12:0), myristic (C14:0) and palmitic (C16:0) SFAs showed a tendency to increase the hematic cholesterol concentration (myristic is more atherogenic) while there's a very high correlation between the sum of three acids (myristic, palmitic and stearic) and the thrombus formation (Ulbricht and Southgati, 1991).

Two distinct indexes were proposed: (i) atherogenic index (IA); and (ii) thrombogenic index (IT). These indexes take in account the different effects that the single fatty acid might have on human health and in particular on probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation. However both these index are subjected to revision in relation to new experimental evidences.

Examining Table 3, in which atherogenic and thrombogenic indexes in adipose tissue under examination are reported, it can be evidenced that the value of both indexes is lower in Se than SPT. Moreover, the comparison with data reported by Franci *et al.* (2005) shows that the values of both indexes are lower in comparison with those observed in *Cinta Senese* (CS), Large White (LW) and LW x CS genetic types.

Table 3. Comparison between mean percentages of atherogenic and thrombogenic index in AAGT *Casertana*

Index	Sampling site				
	Thoracic (SPT)		Lumbar (SPL)		Perirenal
	SI	SE	SI	SE	
IA	0.39	0.32	0.35	0.38	0.5
IT	0.69	0.59	0.65	0.65	0.74

Other unsaturated fatty acids in the present study are represented by 2 of 8 isomers of conjugated linoleic acid (CLA), whose content is lower in perirenal adipose tissue (0.021%) in comparison with subcutaneous adipose tissue (0.26%). The presence of a fair content in CLA is notable since that a such category of PUFA, also at relatively low doses (0.5-1% of the diet) were proved to have an increasingly important role in the interfering positively with immune system and in the prevention of tumour, atherosclerosis and other diseases such as diabetes and obesity (Pariza *et al.*, 2001; Evans *et al.*, 2002).

## Conclusions

Considering all that above explained, the unsaturated fatty acid content of adipose tissue contribute to define food obtained from AAGT under examination as "functional foods", particularly interesting from "nutritional" and "extranutritional" point of view. Unsaturated fatty acids, in addition to being essential components of plasmatic membranes, play a very important role in respiratory mechanics. Therefore pork meat must be considered in the same way of other food of animal and vegetal origin in human nutrition.

Considering the positive recognized influences of fatty acids as regards different diseases, acidic component of pork lipids in human nutrition is considered a "multifactorial" and "multifunctional"

reality, i.e. systemic; this reality must be considered metabolically, and it must be underlined that a healthy and heterogeneous alimentation, able to guarantee a correct supply of main nutrients since young age, represents the best strategy in prevention and curing diseases, therefore conserving a physical, psychical and social welfare optimal but temporarily and spatially dynamic.

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