



Lipid quality indices: Differentiation of suckling lamb and kid breeds reared by traditional sheep farming

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ARTICLE INFO

Article history:

Received 23 January 2012

Received in revised form 11 January 2013

Accepted 16 January 2013

Available online 8 February 2013

Keywords:

Lipids

Fatty acids

Lipid quality indices

Lamb

Kid

Principal component analysis

ABSTRACT

Lipid quality indices (LQI) of six breeds of suckling lambs and kids were determined to evaluate the species and breed effect on intramuscular (IM) and perirenal (PR) fat. Intramuscular fat of all breeds was characterized by high phospholipid and unsaturated fatty acids and low cholesterol content. Perirenal fat mainly consisted of triglycerides. Polyunsaturated/saturated (P/S), ω -6/ ω -3 and hypocholesterolaemic/hypercholesterolaemic fatty acid ratios of IM fat were optimal for all breeds. Specifically, IM fat from Kalarytiko lamb and Skopelos kid presented favourable ω -6/ ω -3 ratio below 3:1. Chios and Lacaune lamb and Skopelos kid IM fat presented the higher P/S ratio and similar to the recommended value of 0.45. Furthermore, atherogenic and most of thrombogenic indices of IM fat remained in desirable levels (<1.0). Significant differences in most lipid quality indices among species and breeds were evident for both IM and PR fat. Principal component analysis (PCA) was used as a tool to cluster the six breeds according to fifteen different LQI. Species and breeds were classified according to their most representative higher values of LQI.

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1. Introduction

Suckling lambs and kids are typical products of the Mediterranean areas and are raised exclusively on maternal milk from birth to slaughter at early age. Depending on each region's market demands, suckling lambs could be high-quality products of strong economic importance (e.g. on Easter period). In suckling lambs, due to their low carcass weight, normally no residual fat is removed since all the soft tissue (fat and muscle) is consumed. Lamb meat

is considered to be a highly nutritious, easily digestible food with a favourable fatty acid composition (Nuernberg et al., 2008). Total lipids and fatty acids, whether in adipose tissue or muscle, contribute significantly to various aspects of quality and organoleptic characteristics of meat and its nutritional value. Specifically, intramuscular, intermuscular and subcutaneous fats significantly affect flavour, juiciness and texture of meat (Juárez et al., 2009). The ratio between polyunsaturated and saturated fatty acids (FA) as well as between ω -6 and ω -3 FA are considered to be two important indices for nutritional evaluation of fat (Department of Health, 1994). However, there is a concern about animal lipid due to its relatively high concentrations of saturated fatty acids (SFA) and low concentration of polyunsaturated fatty acids (PUFA).

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Breed and diet, are the main factors affecting intramuscular fat and depots fatty acid profiles. Also, fat composition is highly influenced by the anatomical location of the fat tissues in lambs' carcasses, due to the differences between internal and external fat depots and to the differences between fatty acid composition of those depots and intramuscular fat. It is generally accepted that the fatty acid composition of the adipose depots of suckling animals depends on length of lactation and the feed consumed as well as the composition of the milk they consume (Velasco et al., 2004).

Breed is a relevant element of lamb and kid production since it may be related to a traditional production system and specific environments. The objectives of the present work were to (a) compare the lipid quality profile (fat content, lipid and fatty acid profile) of intramuscular (IM) and perirenal (PN) fat of two different species (lambs, kids) and six breeds (Lacaune, Kalarytiko, Karagouniko, Chios, Skopelos, Indigenous breed of Greece) of suckling animals, (b) to evaluate the lipid profile according to ten different lipid quality indices (e.g. P/S ratio, ω -6/ ω -3 PUFA ratio) and (c) to differentiate species and breeds with principal component analysis (PCA) based on fifteen different lipid quality indices, calculated by a wide range of different combinations of values of lipid constituents. Furthermore, this study tested the hypothesis that lipid profile indices can be used to categorize species and breeds according to their lipid quality.

2. Materials and methods

2.1. Animals and sampling

The experiment was conducted in Northern Greece in 2009 and 2010 during spring with 20 male animals of each breed (Lacaune, Kalarytiko, Karagouniko and Chios lambs and Skopelos and Indigenous breed kids) reared in different farms, located within an area of 50 km radius, under the same conditions of nutrition and management. These breeds were chosen as the most commercially available breeds of sheep and goat in Greece, representing a high-quality product of major economic

importance. The animals were selected from large size flocks of each breed with a mean 1:15–20 ratio male/female. Throughout the whole experimental period, from birth to slaughter, lambs and kids were single suckled on maternal milk and received no supplementary feed. Mothers' diet was supplied by the same company and was composed mainly of alfalfa hay and concentrate mixture (15.5% crude protein, 2.3% ether extract, 10.9% acid detergent fibre, 18.5% neutral detergent fibre and 6.7% ash) and of the local pasture when available. This diet eliminated any influence of nutritional factors. 10 animals of each breed, aged 40 ± 2 days, were randomly selected and slaughtered during the period from March to April according to the normal procedure at the abattoirs. Carcasses were weighed between 8.0 and 10.0 kg when hot and graded for conformation and external fat classifications. Twenty-four hours post mortem *Longissimus dorsi* muscle was isolated from the right side of the carcasses, cuts were made perpendicularly to the longitudinal axis of the muscle and samples for the IM fat measurement were taken from the centre of each cut; adipose fat samples were taken from the perirenal fat tissue. All samples were vacuum packaged and stored at -20°C until analysis for lipid and fatty acid profile and composition.

The experiments were approved by the Bioethical Committee of the Agricultural University of Athens under the guidelines of Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

2.2. Lipid and fatty acid analysis

Total lipid extraction from obtained samples was performed according to the method of Folch et al. (1957). After phase equilibration the lower chloroform layer (TL) was removed and dried in a rotary vacuum evaporator at 32°C . The extracted lipids were redissolved in chloroform/methanol (9:1, v/v), containing t-butyl-hydroquinone (0.1%) as an antioxidant and finally stored at 0°C until used. Afterwards, representative aliquots of all of the above mentioned samples were evaporated in pre-weighed vials to constant weight to determine the lipid content.

Lipid classes were separated on silicic acid-coated quartz rods, chromarods (Type SIII, 5 mm silica gel-coated quartz rod, Iatron Labs, Tokyo, Japan) and they were quantified using a thin layer chromatography–flame ionization detection system. Iatroscan TLC–FID analysis was performed by an Iatroscan thin-layer chromatograph (Model MK-6 TLC/FID – FPD Analyser Iatron Laboratories, Tokyo, Japan) equipped with a flame ionization detector. Individual lipid classes were quantified as described by Sinanoglou et al. (2011). The lipid standards used for Iatroscan (TLC–FID) were purchased from Sigma Chemical Co. (Sigma–Aldrich Company, Dorset, Great Britain and St. Louis, MO). The data were expressed as a percentage by weight of the total identified lipids. Calibration curves of the standard lipids used were significantly correlated in all cases ($r^2 \geq 0.993$). Reproducibility was tested by spotting the same sample on 10 chromarods and measuring the deviation from the mean. The majority of rods showed a deviation of less than 4%. The reproducibility of daily error was examined and no significant differences ($P > 0.05$) were found.

Fatty acid methyl esters (FAME) of total lipids were prepared according to the procedure described by Sinanoglou and Miniadis-Meimaroglou (1998). Both quantitative and qualitative analysis were performed on an Agilent 6890 Series Gas Chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector, according to the procedure described by Loukas et al. (2010). A capillary DB-23 column ($60\text{ m} \times 0.25\text{ mm i.d. } 0.15\text{ }\mu\text{m film}$) (Agilent Technologies) was used. Fatty acid methyl esters used as GC standards were: lauric acid M-E, cis-5,8,11,14,17-eicosapentaenoic acid M-E, and cis-4,7,10,13,16,19-docosahexaenoic acid M-E (purity $\geq 98\%$) purchased from Sigma Chemical Co. (Sigma–Aldrich Company, UK); Matreya Bacterial Acid Methyl Esters CP™ Mix; Supelco™ 37 Component FAME Mix C4–C24; Supelco PUFA No. 1, Marine Source. The data were expressed as a percentage by weight of the total identified fatty acids. The standard curves for individual FAME were obtained by plotting concentration ratio against area ratio ($r^2 > 0.998$).

2.3. Indices calculations

The ratio between hypocholesterolaemic and hypercholesterolaemic fatty acids (h/H) was calculated according to the formulas suggested by Santos-Silva et al. (2002):

$$\text{C18} : 1\omega 9 + \text{C18} : 2\omega 6 + \text{C20} : 4\omega 6 + \text{C18} : 3\omega 3 + \text{C20} : 5\omega 3 + \text{C22} : 5\omega 3 + \text{C22} : 6\omega 3 \\ \text{C14} : 0 + \text{C16} : 0$$

The peroxidisability index (PI) was calculated according to the formulas proposed by Erickson (1992):

$$(0.025 \cdot \text{monoenes}) + (1 \cdot \text{dienes}) + (2 \cdot \text{trienes}) + (4 \cdot \text{tetraenes}) \\ + (6 \cdot \text{pentaenes}) + (8 \cdot \text{hexaenes}).$$

The atherogenic index (AI) and thrombogenic index (TI) were calculated according to the formulas proposed by Ulbrich and Southgate (1991):

$$\text{AI} = \frac{12 : 0 + (4 \times 14 : 0) + 16 : 0}{\omega\text{-3PUFA} + \omega\text{-6PUFA} + \text{MUFA}}$$

and

$$\text{TI} = \frac{14 : 0 + 16 : 0 + 18 : 0}{0.5\text{MUFA} + 0.5\omega\text{-6PUFA} + 3\omega\text{-3PUFA} + \omega\text{-3PUFA}/\omega\text{-6PUFA}}.$$

The cholesterol index (CI) was calculated according to the Zilversmit (1979) formula:

$$\text{CI} = 1.01 \left(\text{g of SFA } 100\text{ g}^{-1} \text{ of fresh matter} - 0.5 \right. \\ \left. \times \text{g of PUFA } 100\text{ g}^{-1} \text{ of fresh matter} \right) \\ + (0.06 \times \text{mg of cholesterol } 100\text{ g}^{-1} \text{ of fresh matter}),$$

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