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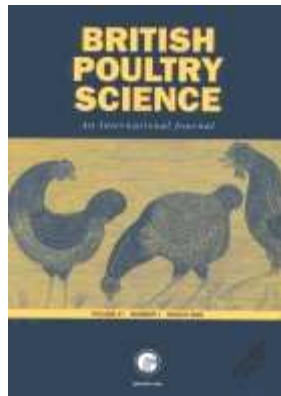
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**Effects of caponization on lipid and fatty acid composition of intramuscular and abdominal fat of medium-growth broilers**

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I have edited the text as there were several parts that required substantial rewriting but I have not been through it in detail. There are also 2 small items that require information from the authors (see comment boxes): there may be others that you raise that can be addressed by the authors at the same time.

Paul Hocking, 7 April 2011

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**Effects of caponisation on lipid and fatty acid composition of intramuscular  
and abdominal fat of medium-growth broilers**

**Short title: Effects caponisation on lipid composition**

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**ABSTRACT**

1. Capons and intact male broilers were used to investigate the effects of caponisation on intramuscular fat and abdominal adipose tissue lipid classes' content and fatty acid profile.
2. Capons had significantly higher total lipid content ( $P<0.05$ ).
3. Neutral lipids were the major fractions in intramuscular and abdominal fat but their proportions differed significantly among groups and tissues ( $P<0.05$ ).
4. The predominant saturated, monounsaturated and polyunsaturated fatty acids in all samples were C16:0, C16:1  $\omega$ -9, C18:0, C18:1  $\omega$ -9, C18:1  $\omega$ -7, C18:2  $\omega$ -6 and C20:4  $\omega$ -6.
5. Caponisation resulted in a significant  $\omega$ -6/ $\omega$ -3, PUFA and PUFA/SFA ratio reduction as well as in a significant atherogenic and thrombogenic indices increase in intramuscular fat ( $P<0.05$ ) without affecting their appropriate value for a healthy diet.

**Keywords:** Total lipids; neutral and polar lipids; fatty acids; capons.

**Abbreviations:** MG, monoglycerides; DG, diglycerides; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; FA, fatty acids; FFA, free fatty acids; l-PC, lyso-phosphatidylcholine; MUFA, monounsaturated fatty acids; NL, neutral lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PhL, phospholipids; PL, polar lipids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Sph, sphingomyelin; TFA, total fatty acids; TG, triglycerides; TL, total lipids.

## INTRODUCTION

Several species of birds are used for commercial production of meat (Mozdziak, 2004), but chickens have become the predominant poultry species produced globally.

The popularity of chicken meat popularity has grown rapidly because the remarkable increase in production efficiency has made it a widely acceptable alternative to traditional red meat, such as beef and pork (Gillipiespie, 2002; Mozdziak, 2004).

Chicken meat is also a low-cost product since modern selection and production techniques have reduced the age at slaughter to approximately 6 weeks with the result that feed conversion efficiency is high (Ravindran, 2004).

In recent times there has been an increase in the demand for more variety and greater quality of poultry meat products. This has led to a reappraisal of the use of traditional practices, such as caponisation (Tor et al., 2002). Caponisation produces a special type of chicken meat since it results in increased overall fatness in terms of abdominal (Cason et al., 1988), subcutaneous and intramuscular fat (Hsieh et al., 2001). **The abdominal adipose tissue is the main fat depot in the body of chickens and can make up approximately 30 % of the mass of major fat depots (Nir et al., 1988). On the other hand, intramuscular fat is located throughout the skeletal muscle and accounts for around 1 % in poultry Pectoralis major muscle.** Nevertheless, fat, whether in adipose tissue or muscle, contributes to important aspects of meat quality (marbling, tenderness, colour) and it is central to the nutritional and sensory values of the meat (Webb and O'Neill, 2008). Specifically, the increased overall fatness in capons enhances flavor, texture and meat juiciness when compared to intact cockerels (Chen et al., 2005).

There have been few investigations of the influence of caponisation on the lipid and fatty acid composition in the carcass and its nutritional value in the human diet.

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Thus, the aim of this research was to determine the lipid and fatty acid composition of intramuscular and abdominal fat of capons and intact cockerels and to evaluate the effect of caponisation on lipid and fatty acid profile.

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## MATERIALS AND METHODS



### Reagents and standards

The lipid standards used were: cholesterol, triolein, lauric acid, oleic acid, linoleic acid, 1,2-diglycerides, monoglycerides, phosphatidylcholine, phosphatidylethanolamine, lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, phosphatidylserine and sphingomyelin standards of the Sigma Chemical Co (Sigma-Aldrich Company, Dorset, Great Britain and St. Louis, MO, USA).

Fatty acid methyl esters used as GC-FID standard mixtures were: Supelco<sup>TM</sup> 37 Component FAME Mix C4-C24, 100 mg Neat. Catalog No:18919-1AMP and Supelco PUFA No.1, Marine Source, 100 mg Neat. Catalog No:47033. All solvents used for sample preparation were of analytical grade and the solvents used for GC and MS analyses were of HPLC grade from Merck (Darmstadt, Germany). Double distilled water was used throughout this work. All reagents used were of analytical grade and they were purchased from Mallinckrodt Chemical Works (St. Louis, MO, USA) and from Sigma Chemical Co (Sigma-Aldrich Company, UK).

### Animal Management and Experimental Design

The experiment was conducted in the experimental facilities of the Department of Animal Science and Aquaculture of the Agricultural University of Athens. Feather-sexed male medium-growing broilers (Redbro, Hubbard SAS, France) were purchased from a local hatchery on day zero. On arrival, birds were wing-marked,

1 weighed and randomly assigned to solid  ~~hybrid~~ pens (200 x 100 x 180 cm; depth x  
2 width x height) with wheat straw bedding. The treatments consisted of 75 intact males  
3 and 50 capons. Duration of the experiment was 18 weeks. Lighting program was as  
4 follows: 24 hours of light for the first two days, 23L-1D from day 3 until the 3<sup>rd</sup> week  
5 of age, 18L- 6D from 3<sup>rd</sup> to 8<sup>th</sup> week and 16L- 8D until the end of the experiment.  
6 Ambient temperature was gradually decreased from 32° C on day zero to 20° C on  
7 day 30 and was then kept within the range of 18-20° C throughout the experiment.  
8 Food and water consumption was *ad libitum*. The birds were fed a commercial starter  
9 diet in crumbs from day 1 to day 35 while for the rest of the experimental period they  
10 consumed a commercial finisher diet in crumbs. The experiment was conducted from  
11 November to March 2007. 

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24 The experiment was approved by the bioethical committee of the Agricultural  
25 University of Athens under the guidelines of “Council Directive 86/609/EEC  
26 regarding the protection of animals used for experimental and other scientific  
27 purposes”.

### 34 Caponisation

35 Caponisation was performed at 3 weeks of age. The birds selected for surgery  
36 were deprived of feed and water for 24 and 12 hours, respectively, before the  
37 procedure. Anaesthesia was performed using Xylazine (Rompun, Bayer HealthCare,  
38 Germany) and Ketamine (Ketaset, Fort Dodge, USA) in doses of 1.5 and 23 mg/kg  
39 live weight, respectively. The mean ( $\pm$  S.E.) live weight of the chicks was  $650 \pm 19$  g.  
40 After feather removal, the incision area was prepared with povidone iodine 10%  
41 (Betadine solution, Mundipharma, Switzerland) and a 1.5 cm incision was performed  
42 between the two last ribs. Incomplete caponised chickens (slips) rates were 6.4 %.

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2 These birds were identified by the color and size of the combs and were removed  
3  
4 from the experiment. Mortality rates for the whole experiment were 3.4 %.  
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### 8 **Slaughter procedure**

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10 At 18 weeks of age, ten chickens per group were randomly selected and they  
11 were fasted 16 hours before slaughter. They were electrically stunned (120 V/ 50 Hz  
12 for 5 sec), killed by decapitation and left to bleed for approximately 1 min.  
13  
14 Afterwards, they were passed through a warm scalding vessel (60° C for 2 min), a  
15 plucker (2 min) and were manually eviscerated. After primary cooling on air for  
16 approximately 1 hour, they were stored at 4° C overnight.  
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### 23 **Total lipids extraction**

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25 Total lipids extraction was performed in Pectoralis major muscle and abdominal  
26 fat samples according to the method of Folch et al. (1957). After phase equilibration  
27 the lower chloroform layer (TL) was removed and dried in a rotary vacuum  
28 evaporator at 32°C. The extracted lipids were redissolved in chloroform/methanol (9:1  
29 v/v) and finally stored at 0°C until used. To prevent oxidation t-butyl-hydroquinone,  
30 at a concentration of 100 mg/l, was added to all samples during preparation.  
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38 Afterwards, representative aliquots of 10-12 mg of total lipids were evaporated in pre-  
39 weighed vials to constant weight to determine the lipid content.  
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### 43 **Iatroscan analysis of neutral and polar lipids**

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45 Lipid classes were separated on silicic acid-coated quartz rods Chromarods  
46 (Type SIII) (silica gel-coated quartz rod. Iatron Labs, Tokyo, Japan) and then  
47  
48 quantified using a thin layer chromatography–flame ionisation detection system  
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2 (TLC-FID) (Sinanoglou et al., 2009). TLC-FID analysis was performed by an  
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4 Iatroscan thin-layer chromatograph (Model MK-6 TLC / FID - FPD Analyser, Iatron  
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6 Laboratories, Tokyo, Japan) equipped with a flame ionization detector and connected  
7  
8 to a personal computer for collecting the chromatograms. Operation conditions for the  
9  
10 Iatroscan were 160 mL min<sup>-1</sup> hydrogen flow, 2 L min<sup>-1</sup> air flow and 30 s/chromarod  
11  
12 scanning speed. A 1 µL Hamilton syringe (Hamilton Co., Reno, USA) was used to  
13  
14 spot standards and samples. The rods were developed with a one- or two-solvent  
15  
16 system. The neutral solvent system consisted of n-H:DE:FA (60:15:1.5 by vol.) as  
17  
18 well as the polar solvent system of C:M:W (50:20:2. by vol.).

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20 The determination of weight percentages of individual lipid classes was  
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22 achieved using the standard curves obtained for each authentic standard (0.15 to 0.25  
23  
24 mg/ml per lipid) under the same chromatogram conditions and development  
25  
26 temperature. The correlation coefficients for the calibration curves of thirteen lipid  
27  
28 classes were in all cases higher than 0.998.

### 31 32 33 **Gas chromatography analysis of fatty acid methyl esters**

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35 Fatty acid methyl esters (FAME) of total lipids were prepared according to the  
36  
37 procedure described by Sinanoglou and Miniadis-Meimaroglou (1998). Both  
38  
39 quantitative and qualitative analyses were performed on an Agilent 6890 Series Gas  
40  
41 Chromatograph equipped with a flame ionization detector. DB-23 capillary column  
42  
43 (60m × 0.25mm i.d., 0.15µm film) [50%-Cyanopropyl)-methylpolysiloxane] (Agilent  
44  
45 Technologies, Catalogue No: 122-2361) was used. Helium was used as a carrier gas.  
46  
47 The injector and detector temperature were 250°C and 260°C respectively. The  
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49 temperature was programmed at 100°C for 0 min, raised from 100 to 150°C by a rate  
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51 of 10°C min<sup>-1</sup> and held constant at 150°C for 0 min, then raised from 150 to 195°C by  
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2 a rate of 2°C min<sup>-1</sup> and held constant at 195°C for 5 min, then raised from 195 to  
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4 210°C by a rate of 1°C min<sup>-1</sup> and held constant at 210°C for 0 min and finally raised  
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6 from 210 to 240°C by a rate of 10°C min<sup>-1</sup> and held constant at 240°C for 5 min. The  
7  
8 duration of the analysis was 55.50 min (Sinanoglou et al., 2009).  
9

10 Individual fatty acids were quantified according to the formula:

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12  $\mu\text{g (FA)} = \text{FAME peak area} \times \text{FAME response factor}$ , where FAME response factors  
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14 were determined according to the FAME standard chromatograms under the same  
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16 chromatographic conditions, and were identified by comparison of their retention  
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18 times to those of the standard mixtures.  
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### 23 Statistical analysis

24 All measurements were obtained (at least) in triplicate and values were averaged  
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26 and reported along with the standard error (s.e.). Data were analyzed using multifactor  
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28 ANOVA fitting the treatment and the origin of fat as fixed factors. Multiple  
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30 comparisons were conducted with the Tukey's honestly significant difference test. All  
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32 data were analyzed with the SPSS-10 statistical software designed for Windows  
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34 (Chicago, IL, USA).  
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## 39 RESULTS

### 40 Total, neutral and polar lipid content

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42 Total (TL, g/100g of muscle or adipose tissue), neutral (NL) and polar (PL)  
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44 lipids (mg/100mg of TL) of intramuscular fat and abdominal adipose tissue of capons  
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46 and intact males are presented in Table 1.  
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51 (Table 1 presented here)  
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TL content was significantly lower in the intramuscular fat than in the abdominal adipose tissue ( $P<0.05$ ). In both intramuscular fat and abdominal adipose tissue, TL mainly consisted of NL, but in the intramuscular fat the percentage of PL was significantly higher than in the abdominal adipose tissue.

Capons had a significantly higher content of TL in intramuscular fat and the abdominal adipose tissue in comparison to intact males ( $P<0.05$ ). Capons also had a higher NL percentage in the intramuscular fat while the opposite result was observed for the abdominal adipose tissue.

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### Neutral and polar lipid profile

Abdominal NL of capons and intact males consisted mainly of TG (>99.5% of total NL) while PL mainly consisted of PC (>90.0% of total PL) and PE (>8.5% of total PL). Differences between the groups were not significant (data not shown). The neutral and polar lipid profile of intramuscular fat of capons and intact males is presented on Table 2. Intramuscular NL mainly consisted of TG followed by cholesterol, FFA, DG and MG. In the intramuscular PL, PC had the highest percentage followed by PE, Sph, PS and l-PC.

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(Table 2 presented here)

The intramuscular TL of capons contained a significantly higher TG percentage ( $P<0.05$ ) compared to those of intact males, while the opposite result was observed for FFA, cholesterol, MG, and PL percentages.

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## Fatty acids

The fatty acid (FA) profile of TL isolated from intramuscular and abdominal fat of capons and intact males is shown in Table 3. The carbon chain length of the identified fatty acids ranged from 14C to 24C and the number of double bonds from 0 to 6.

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The major fatty acids of both the abdominal and intramuscular TL of capons and intact male chicks were oleic (C18:1 $\omega$ -9), palmitic (C16:0), linoleic (C18:2 $\omega$ -6) and palmitoleic (C16:1 $\omega$ -9) acid. Significantly higher concentrations of arachidonic acid (C20:4 $\omega$ -6) were found in intramuscular compared to abdominal TL.

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(Table 3 presented here)

C18:2 $cis$ -9, $trans$ -11 comprised less than 0.1% of total fatty acids across both abdominal adipose tissue and intramuscular fat. Intramuscular TL were higher in SFA, PUFA,  $\omega$ -3 and  $\omega$ -6 fatty acids while abdominal TL were higher in MUFA. This resulted in an increased SFA/MUFA ratio in intramuscular in contrast to abdominal TL while the opposite was observed for PUFA/SFA and  $\omega$ -6/ $\omega$ -3 ratios.

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Intramuscular TL of capons, in comparison with intact males, had significantly higher percentages of SFA and  $\omega$ -3 FA and lower PUFA and  $\omega$ -6 FA. There was no statistically significant difference in MUFA percentages between the groups.

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Specifically, TL from intramuscular fat of capons were significantly higher in myristic (C14:0), palmitic (C16:0), stearic (C18:0) and arachidonic (C20:4  $\omega$ -6) acid

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and significantly lower in linoleic (C18:2  $\omega$ -6) acid than intact males, resulting thus in significantly lower  $\omega$ -6/ $\omega$ -3 and C16:0/C18:0 ratios.

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With respect to the abdominal adipose tissue, capons had lower proportions of SFA, PUFA,  $\omega$ -3 and  $\omega$ -6 fatty acids and higher proportions of MUFA. The SFA/MUFA and PUFA/SFA ratios were not different between the experimental groups while the  $\omega$ -6/ $\omega$ -3 ratio was higher in capons.

## DISCUSSION

It is well established that caponisation results in increased body fat accumulation in terms of both abdominal (Cason et al., 1988; Tor et al., 2002; Hsu and Lin, 2003; Chen et al., 2005; Chen et al., 2006a; Chen et al., 2006b; Miguel et al., 2008) and intramuscular fat (Tor et al., 2002; Miguel et al., 2008). The same phenomenon has been observed for bovine L. dorsi muscle intramuscular fat (Hood and Allen, 1971) but not for porcine L. dorsi muscle, even though the backfat thickness was higher in castrates in comparison to intact male pigs (Hogberg et al., 2004).

The relative importance of intramuscular fat, as far as the final product is concerned, is much higher in poultry since most of the abdominal adipose tissue is discarded during standard slaughtering conditions. To our knowledge, there is only one reference in the literature that addresses the effects of caponisation on lipid composition. In that work, Sirri et al. (2009) reported that caponisation increased SFA content of Pectoralis minor muscle while it did not affect MUFA, PUFA,  $\omega$ -3 and  $\omega$ -6 content. In mammals, castration was reported to significantly influence bovine L. dorsi intramuscular fatty acid composition since the SFA proportion was higher in steers whereas PUFA was higher in bulls (Clemens et al., 1973; Eichhorn et al., 1985). Moreover, steers had a lower  $\omega$ -6/ $\omega$ -3 ratio, which is considered to have beneficial effects on human health (Monteiro et al., 2006). Even in comparison to

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heifers, the intramuscular fat of steers was found to have higher and lower proportions respectively of SFA and unsaturated fatty acids (Waldman et al., 1968; Terrell et al., 1969; Zembayashi et al., 1995).

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In porcine L. dorsi intramuscular fat the effects of castration on fatty acid composition are reported to be similar. Barrows had higher proportions of SFA, especially C16:0 and C18:0, while boars had higher proportions of MUFA, PUFA, n-6 and n-3 fatty acids (Hogberg et al., 2004). Moreover, in several studies, the level of unsaturation of the intramuscular fat is higher in gilts than in castrated males (Hogberg et al., 2001, 2002; Hogberg et al., 2003; Nilzen et al., 2001). Moreover, Hogberg et al. (2003) found a positive correlation between SFA and IMF and a negative correlation between unsaturated FA and IMF. Consequently, the increased level of SFA in the intramuscular fat of capons was an indirect effect of the increased intramuscular fat accumulation due to caponisation.

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The ratio of PUFA to SFA is considered to be important in relation to the nutritional value of foods for human health. In the human diet, the minimum recommended value for PUFA/SFA ratio is 0.45–0.65 (Department of Health, 1984) and lower ratios in the diet as a whole may result in increased incidents of cardiovascular disease. Thus, caponisation included a PUFA reduction and a replacement of PUFA with SFA, but without affecting the positive PUFA/SFA ratio. High levels of MUFA were observed in both intramuscular fat and abdominal adipose tissue of the experimental groups as the distribution pattern of the sum of fatty acids in TL was: MUFA>SFA>PUFA. MUFA of the *cis*-configuration, compared with SFA, are hypocholesterolemic with the advantage of not reducing HDL-cholesterol, which protects against coronary heart disease (CHD) (Mattson and Grundy, 1985).

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Abdominal TL contained only traces of arachidonic acid (C20:4  $\omega$ -6) in comparison to intramuscular TL. Arachidonic acid is synthesized from linoleic acid (Machlin and Gordon, 1961). This indicates that capons and intact males probably synthesized arachidonic acid and deposited a lower percentage of it in the lipid rich tissues (abdominal fat) than in the meat. In contrast to our results, Sirri et al. (2009) reported that arachidonic acid in the intramuscular fat of Pectoralis major muscle significantly increased from capons to slips and to cocks, and this difference was attributed to the inhibition of  $\Delta^6$ -desaturase by the caponisation. In our study, capon intramuscular fat contained significantly higher arachidonic acid and significantly lower linoleic acid than intact males, which might be associated with the commercial diet and/or the metabolism of the birds. The absence or the trace amounts of C20 and C22 polyunsaturated fatty acids (PUFA) in abdominal TL resulted from the low proportion of PL (<5%) in the TL fraction and the possible failure of abdominal adipose tissue to incorporate these fatty acids into the triglycerides.

One method of estimating food quality, in terms of lipids, is the use of atherogenic and thrombogenic indices, as proposed by Ulbricht and Southgate (1991).

The atherogenic index of abdominal fat for capons and intact males respectively was 0.35 and 0.37 and the indexes for intramuscular fat were 0.38 and 0.43. The thrombogenic index of capons and intact males for abdominal fat was 0.68 and 0.70 and for intramuscular fat it was 0.71 and 0.83. Caponization, in general, induced an increase in both indices but remained lower than the appropriate value for a healthy diet recommended by Ulbricht and Southgate (1991). Žlender et al. (2001) reported an atherogenic index of 0.49 and 0.51 for the semitendinosus and triceps brachii muscles of Simmental and Brown bulls. Vacca et al. (2008) reported that indices of atherogenicity and thrombogenicity in muscles of Mouflon  $\times$  Sarda and Sarda

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suckling lambs varied between 0.9 and 1.1, whereas Valfre et al. (2003) reported atherogenic indexes ranging from 0.25 to 0.45 and thrombogenic indexes of 0.45 to 1.35 for fish meat.

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Cholesterol is a nutritionally important component of meats. After having taken into consideration the cholesterol values of capons and intact males intramuscular fat, their contribution to the recommended maximum cholesterol intake can be calculated through the consumption of 100g of capon and intact male muscular tissue. This contribution represented 14% and 11%, respectively, of the recommended maximum cholesterol intake (300 mg per day; De Backer et al., 2003).

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On comparing capons and intact males, the results indicated that caponisation had a considerable effect on the total fat and fatty acid content. As consumers are looking for a diet with low fat and high levels of PUFA, caponisation indicated an improved quality of meat, including a good fatty acid profile, low cholesterol content and PUFA:SFA ratio, satisfactory atherogenic and thrombogenic indexes, and a reduction in the ratio of  $\omega$ -6/ $\omega$ -3 fatty acids. However, the higher production cost due to the caponization procedure would have to be covered by the premium prices that these products have in the market.

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Besides the promising meat quality of capons there is also an ethical concern regarding the practical administration of the caponization technique on everyday practice. In the more distant past, capons were produced by surgery without anesthetic, which was banned for welfare reasons, then later by hormonal means, which was banned because of residues in the meat. The surgical technique could still be unacceptable in many countries because a degree of distress is inevitable even when performed under anesthesia. In time, of course, it may be that capons can be

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1  
2 produced without stress by genetic modification or by chemical means without the  
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4 hormonal residues, but that is for the future.  
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