**ARTICLE** 

# Effect of Growth on Fatty Acid Composition of Total Intramuscular Lipid and Phospholipids in Ira Rabbits

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#### Abstract

The changes in fatty acid composition of total intramuscular lipid and phospholipids were investigated in the longissimus dorsi, left-hind leg muscle, and abdominal muscle of male Ira rabbits. Changes were monitored at 35, 45, 60, 75, and 90 d. Analysis using gas chromatography identified 21 types of fatty acids. Results showed that the intramuscular lipid increased and the intramuscular phospholipids (total intramuscular lipid %) decreased in all muscles with increasing age (p<0.05). An abundant amount of unsaturated fatty acids, especially polyunsaturated fatty acids, was distributed in male Ira rabbits at different ages and muscles. Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and arachidonic acid (C20:4) were the major fatty acids, which account to the dynamic changes of the n-6/n-3 value in Ira rabbit meat.

Keywords: Ira rabbit, fatty acid, total intramuscular lipid, intramuscular phospholipids, growth

#### Introduction

In the last 50 years, the world production of rabbit meat has increased by 2.5-fold to 1.6 million tons in 2009. China is currently the world's leading producer of rabbit meat (700,000 t/year). Italy (230,000 t/year), Spain (74,161 t/year), and France (51,400 t/year) are the main rabbit-meat producers in Europe (FAOSTAT). Rabbit meat offers excellent nutritive and dietetic properties (Hernàndez and Gondret, 2006) and is characterized by very low monounsaturated fatty acids (MUFA) levels, high levels of polyunsaturated fatty acids (PUFA) and n-3 fatty acids. The meat is also a significant source of vitamin B family (vitamins B2, B5, B6, B3, and B12) and has very high phosphorus contents. Compared with other animal meats, rabbit meat has lower fat levels. The protein content of rabbit meat offers essential amino acids (lysine and tryptophan), especially at the longissimus dorsi muscle, which also has high digestibility (Hernández, 2008). Rabbit meat can be used to produce specific functional food (Dalle Zotte and Szendrő, 2010; Hernández, 2008) since its good properties (e.g., fatty acid profile, mineral, and vitamin contents) and can be modified and further enriched via feeding (Bianchi *et al.*, 2009; Combes and Dalle Zotte, 2005; Dalle Zotte, 2002; Gigaud and Combes, 2008; Hernández and Gondret, 2006; Maertens *et al.*, 2008; Molette *et al.*, 2009).

Nowadays, current research is directed toward developing feeding strategies to increase the nutritional value of rabbit meat as a "functional food" by including vitamins, antioxidants and essential fatty acids in rabbit diets and assessing their effects on quality of both raw and stored/ processed meat (Cavani et al., 2009; Petracci et al., 2009). However, limited information is available on the effect of ages and muscles on fatty acid profile of total intramuscular lipids and phospholipids from one breed of rabbit. Several factors, such as age (or weight), gender, genotype, and other external factors (transportation, slaughter and season), can influence both the quantity and quality of lipids in animal products (Kouba and Mourot, 2011). The lipid composition, especially the amount of intramuscular phospholipids in meat, is an important factor for flavor development and nutritive quality of freshcooked and dry-cured meat products (Cappelli and Vannucchi, 2005; Gray et al., 1996). Phospholipids are found in significant amount in muscles, which contain a high PUFA content to perform its function as a constituent of cellular membranes (Wood et al., 2008).

Ira rabbits are imported from France and are of high

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breeding efficiency. However, limited information is available on the fatty acid profile of total intramuscular lipids and phospholipids during the growth of Ira rabbits. Therefore, this study was conducted to examine the changes of fatty acid composition of total intramuscular lipid and phospholipids by gas chromatography to provide a database for the subsequent in-depth study of Ira rabbits.

#### **Materials and Methods**

#### Animals and diets

A total of 150 35-d-old weaned male Ira rabbits (30 per age) were provided by College of Animal Science and Technology, Southwest University. The ingredients and proximate chemical composition of the diet were shown in the Table 1. They were maintained in a closed building under natural environmental conditions in individual wire mesh cages, equipped with metal troughs and automatic nipple drinkers. The rabbits had free access to feed and

Table 1. The ingredients and proximate chemical composition of the diet

Item	Diets						
Ingredients	Proportion (%)						
Corn	24.2						
Wheat bran	19						
Soybean meal	10.82						
Alfalfa meal	36						
Corn germ cake	4						
Rapeseed	3						
Powder	0.5						
Dicalcium	0.8						
Lysine	0.07						
Methionine	0.11						
Salt	0.5						
Premix <sup>a</sup>	1						
Nutrition	Proportion (%)						
Dry matter	89.8						
Crude protein	16.0						
Fat	3.3						
Lysine	0.7						
Methionine	0.6						
Calcium	0.95						
Phosphorus	0.59						
Acid detergent fiber	33.2						
Neutral detergent fiber	21.4						
Digestible energy <sup>b</sup>	10.5 MJ/kg						

 $<sup>^{\</sup>rm a}$ The premix contains (per kg of diet): Vitamin A, 10000 IU; Vitamin D\_3, 1000 IU; Vitamin E, 30 mg; Vitamin K, 1 mg; Vitamin B\_1, 1 mg; Vitamin B\_2, 3.5 mg; Vitamin B\_6, 2 mg; Vitamin B\_{12}, 0.01 mg; niacin, 50 mg; folic acid, 0.3 mg; choline, 1000 mg; Zn, 30 mg; Cu, 5 mg; Mn, 15 mg; Fe, 30 mg; I, 1 mg.

water.

The rabbits were bred under similar production system and slaughtered at the age of 35, 45, 60, 75, and 90 d in a local commercial slaughterhouse. During the experiment, the slaughter weights were recorded as 1.00±0.06, 1.31±0.01, 2.15±0.12, 2.57±0.08, 3.18±0.07 kg. The facilities of the slaughterhouse met the requirements of the Institute of Animal Care and Use Committee. After 24 h postmortem, the longissimus dorsi muscle (LD), left-hind leg muscle (LL) (left biceps femoris muscle), and abdominal muscle (AM) (ventral musculus) of the carcass were removed and immediately vacuum-packed and frozen at 20°C until treatment.

## Total intramuscular lipid content and fatty acid composition analysis

Total intramuscular lipids were extracted according to Folch et al. (1957). Total lipid content was measured by weighing the lipid extracts after solvent evaporation. Fractions of intramuscular phospholipids were prepared with silica cartridges (Sep-Pack, Waters, USA) by using the method of Juaneda and Rocquelin (1985). Phospholipids were quantified by phosphorous determination (Bartlett, 1959). The total lipids and phospholipids were methylated with boron fluoride-methanol (Sigma Aldrich) according to Morrison and Smith (1964). The fatty acid methyl esters were analyzed by a QP-2010 gas chromatograph (Shimadzu, Japan) equipped with a flame ionization detector and a split injector. One microliter of fatty acid methyl esters was injected in split mode (5:1) onto a Rtx-Wax capillary column (Restek, Bellefonte, USA; 30  $m \times 0.25$  mm id  $\times 0.25$  µm film thickness). The temperature of the column was programmed as follows: 1 min at 140°C, increments of 8°C/min to 180°C and held at 180°C for 2 min; increments of 3°C/min to 210°C; and increments of 5°C/min to 230°C and held at 230°C for 10 min. The temperature of the injector and the detector were both 250°C. The flow rate of the carrier gas (N<sub>2</sub>) was 1.5 mL/min. Fatty acids were identified by comparing the retention time of the samples with those of the standards (Sigma). Results were expressed as percent of the total fatty acid methyl esters.

#### Statistical analysis

Statistical Analysis System (1996) was used to determine the means, standard errors, and analysis of variance. Duncan's multiple range test was used to compare differences among means. Values at p<0.05 were considered significant.

<sup>&</sup>lt;sup>b</sup>Digestible energy (kcal/kg DM) = TDN  $\times$  4400 (NRC, 1985).

### **Results and Discussion**

### Effect of age and muscle on total intramuscular lipid and phospholipids content in male Ira rabbits

Variation of intramuscular lipid content

Fig. 1 shows the effect of age on total intramuscular lipid content among the muscles from male Ira rabbits. The total intramuscular lipid content (muscle weight %) of LD, LL, and AM significantly increased with increasing age of rabbits (p<0.05). The intramuscular lipid content among muscle sections (LD, LL, and AM) in the male rabbits increased from 0.77, 1.16, and 2.41 g/100 g muscle at 35 d to 1.21, 1.66, and 5.16 g/100 g muscle at 90 d, respectively. Among the three muscles, AM had the highest intramuscular lipid content, followed by LL and LD at each tested age. This result is consistent with the study of Hernández and Dalle Zotte (2010), who reported that the leanest cut of meat in the rabbit carcass was the loin, besides the hind leg was the most quantitatively important cut because of its low lipid content compared with other muscles. Therefore, lipid content depends greatly on the age and muscle of the rabbit. During the 35 d to 90 d Ira rabbit growth period, the total intramuscular lipid deposition of AM was significantly higher than LL and LD, of which the lipid content of the three sections increased faster during the 60 d to 75 d period than during the other growth stages. Thus, this period was characterized with the most expressed intramuscular fat deposition of male Ira rabbits. However, there was no significant difference in intramuscular lipid content of LD between 60 d (bc) and 75 d (ab).

Variation of intramuscular phospholipids content Fig. 2 shows that there is no significant reduction in phospholipid content of the muscles. Only the percentage of phospholipid in total muscle lipid decreases with age (p<0.05) (Fig. 3). This result may be attributed to the increasing deposition of triglycerides in the adipose tissues (Raes *et al.*, 2004).

During the 35 d to 90 d growth period, LL showed the fastest reduction in phospholipid content, which was significantly higher than that in the AM and LD. In the 35 d to 45 d growth period, the reduction in phospholipids on all three muscles was more obvious than in the other growth stages. During the 35 d to 90 d growth period, the intramuscular phospholipids content decreased from 43.47, 53.81, and 42.21 g/100 g intramuscular lipids at 35 d to 26.84, 28.89, and 25.40 g/100 g intramuscular lipids at 90 d, respectively. Among the muscles at different ages, the LL total lipid accounted for the highest relative phospholipid content, followed by LD and AM. However, compared with the absolute percentage of intramuscular phospholipids (muscle weight %), the change of intramuscular

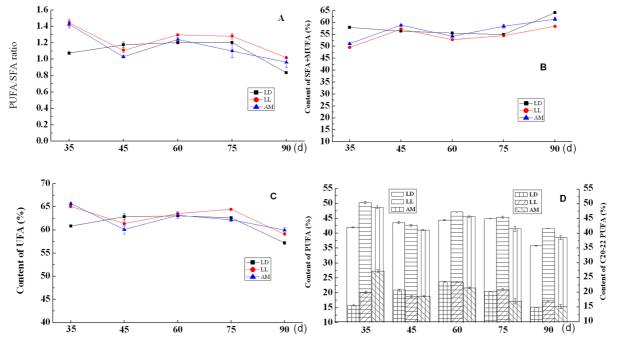


Fig. 1. Comparison of intramuscular lipid content (muscle weight %) in male Ira rabbits at different ages and muscles. (LD, Longissimus dorsi; LL, left-hind leg muscle; and AM, abdominal muscle)

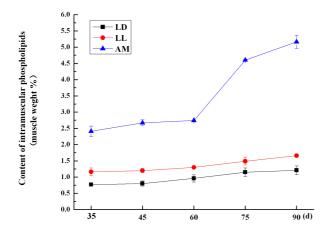


Fig. 2. Comparison of intramuscular phospholipids content (muscle weight %) in male Ira rabbits at different ages and muscles. (LD, Longissimus dorsi; LL, left-hind leg muscle; and AM, abdominal muscle)

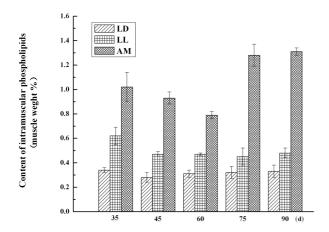


Fig. 3. Comparison of intramuscular phospholipids content (lipid weight %) in male Ira rabbits at different ages and muscles. (LD, *Longissimus dorsi*; LL, left-hind leg muscle; and AM, abdominal muscle)

phospholipids among the three muscles of Ira rabbit were not significant (Fig. 1). Therefore, the content of phospholipids in muscle was relatively stable during the 35 d to 60 d growth period. Meanwhile, among the three muscles, AM accounted for the maximum absolute percentage of intramuscular phospholipids (muscle weight %), followed by LL and LD.

## Effect of age and muscle on fatty acid composition of total intramuscular lipid

Table 2 showed the comparative total intramuscular lipid in LD, LL, and AM of male Ira rabbit meat of different ages. All the total lipids obtained high unsaturated fatty acid (UFA) proportion (the sum of PUFA and MUFA),

especially PUFA. In rabbit meat, UFA represents around 60% of the total FA. By comparison, PUFA comprises 32.5% of the total FA, which is the highest among other meats, such as pork (13.8%), beef (8.79%), veal (13.3%), and chicken (27.4%) (Salma et al., 2007; Wood et al., 2008). The result of this previous study was consistent with our present investigation. Saturated fatty acids (SFA) were mainly composed of palmitic acid (C16:0) and stearic acid (C18:0). MUFA consisted of oleic acid (C18:1). PUFA, such as linoleic acid (C18:2n-6) and arachidonic acid (C20:4), were observed at high levels. In the total intramuscular lipid, C18:2n-6 represents 22±4.7% of the total FAs (average of 20 referenced studies, all meat portions combined together). Among the long-chain (C20-22) PUFAs, the eicosapentaenoic acid (EPA, 20:5n-3) percentage in rabbit loin meat constitutes 0.15±0.12%, and docosahexaenoic acid (DHA, 22:6n-3) is 0.31±0.31% of the total FAs (Hernández and Dalle Zotte, 2010). These reported data are lower than those of the present study. There is an increasing recognition of the health benets of polyunsaturated fatty acids (PUFA), and of n-3 fatty acids in particular, because these fatty acids are essential for humans (Conquer and Holub, 1998; Kouba et al., 2011; Simopoulos, 2001).

With increasing age of rabbits, the percentage of SFA in LD and LL did not increase significantly (p>0.05), whereas the SFA proportion from AM increased significantly (p<0.05). This result is attributed to the changes of C16:0 content in total intramuscular lipids, corresponding to the significant reduction of PUFA in the muscles (p<0.05) and the significant increase of MUFA in LD and LL (p<0.05). In addition, during the growth of Ira rabbits, a significant reduction of PUFA/SFA ratio (1.10 to 0.90, 1.10 to 0.94, 0.98 to 0.80) and a significant increase in SFA + MUFA (57.94% to 65.06%, 55.94% to 61.61%, 60.74% to 66.20%) were observed in the LD, LL and AM, respectively.

By comparing the FA profile in LD, LL, and AM, we determined that lauric acid (C12:0), heptadecanoic acid (C17:0), myristoleic acid (C14:1), C18:2n-6, C20:4n-6, eicosapentaenoic acid (EPA, 20:5n-3), and DHA were decreased significantly (p<0.05). C16:0, palmitoleic acid (C16:1n-7), eicosadienoic acid (C20:2n-6), and eicosatrienoic acid (C20:3n-6) were increased significantly (p<0.05), whereas apparent differences were not observed in cis-10-heptadecenoic (C17:1) (p>0.05). Overall, in terms of C18:1n-9 proportion, LD was sequentially higher than AM and LL (p<0.05). For the C18:2n-6 proportion, LL had the highest percentage (30.46% to 27.11%), whereas

Table 2. Composition of the fatty acids of intramuscular lipid (%) of male Ira rabbits at different ages and muscles<sup>a</sup>

LL AM  0.06± 0.07± 0.01bc 0.01c  1.20± 1.78± 0.01bc 0.04b  0.33± 0.38± 0.02c 0.01b  0.39± 0.50±
$\begin{array}{ccc} 0.01^{bc} & 0.01^{c} \\ 1.20 \pm & 1.78 \pm \\ 0.01^{b}c & 0.04^{b} \\ 0.33 \pm & 0.38 \pm \\ 0.02^{c} & 0.01^{b} \\ 0.39 \pm & 0.50 \pm \\ \end{array}$
$\begin{array}{ccc} 1.20 \pm & 1.78 \pm \\ 0.01^b c & 0.04^b \\ 0.33 \pm & 0.38 \pm \\ 0.02^c & 0.01^b \\ 0.39 \pm & 0.50 \pm \end{array}$
$\begin{array}{ccc} 0.01^b c & 0.04^b \\ 0.33 \pm & 0.38 \pm \\ 0.02^c & 0.01^b \\ 0.39 \pm & 0.50 \pm \end{array}$
$\begin{array}{ccc} 0.33 \pm & 0.38 \pm \\ 0.02^c & 0.01^b \\ 0.39 \pm & 0.50 \pm \end{array}$
$0.02^{c}$ $0.01^{b}$ $0.39\pm$ $0.50\pm$
0.39± 0.50±
$0.03^{\circ}$ $0.16^{\circ}$
25.61± 26.43±
0.01° 0.51° 3.07± 4.34±
$0.04^{a}$ $0.05^{a}$
0.04   0.03 $0.33\pm   0.34\pm$
$0.01^{\circ}$ $0.01^{\circ}$
0.27± 0.31±
$0.02^{\circ}$ $0.03^{\circ}$
11.59± 12.84±
$0.76^{b}$ $0.69^{a}$
17.13± 17.53±
$0.66^{a}$ $1.00^{a}$
1.28± 1.20±
$0.06^{a}$ $0.02^{a}$
27.11± 25.03±
$0.02^{d}$ $0.63^{c}$
0.99± 1.46±
$0.02^{b}$ $0.04^{b}$
$0.04\pm 0.06\pm$
$0.01^{\rm b}$ $0.01^{\rm b}$
$0.30\pm 0.42\pm$
$0.01^{a}$ $0.07^{a}$
0.96± 0.73±
$0.01^{a}$ $0.02^{a}$
$0.73\pm 0.55\pm 0.02h$
0.01 <sup>ab</sup> 0.02 <sup>b</sup>
5.54± 3.87± 0.01 <sup>e</sup> 0.08 <sup>e</sup>
1.79± 1.32±
$0.01^{d}$ $0.02^{d}$
0.01  0.02 $0.76\pm  0.48\pm$
$0.70^{\circ}$ $0.48^{\circ}$ $0.02^{\circ}$ $0.04^{\circ}$
0.51± 0.35±
0.01° 0.02°
39.22± 42.02±
0.73 <sup>cd</sup> 1.93 <sup>b</sup>
38.39± 33.80±
$0.10^{\rm d}$ $0.86^{\rm d}$
22.39± 24.18±
$0.74^a$ $2.79^a$

 $<sup>^</sup>a\mbox{Results}$  were expressed as means  $\pm$  PE, data were means of three replicates.

LD had the lowest percentage (28.58% to 24.86%). The C20:4n-6 proportion in AM was significantly lower than

that in LD and LL (p<0.05). The other major components did not differ significantly. During the growth of Ira rab-

<sup>&</sup>lt;sup>b</sup>Values of LD, LL and AM in the same row with different letters were significantly different, respectively (*p*<0.05).

cSFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids.

<sup>&</sup>lt;sup>d</sup>LD, *Longissimus dorsi*; LL, left-hind leg muscle; AM, abdominal muscle.

Table 3. Composition of the fatty acids of intramuscular phospholipids (%) of male Ira rabbit at different ages and muscles<sup>a</sup>

		35 d			45 d			60 d	,		75 d			90 d	
Fatty acids	LD	LL	AM	LD	LL	AM	LD	LL	AM	LD	LL	AM	LD	LL	AM
C12:0 <sup>b</sup>	0.10± 0.02 <sup>a</sup>	0.05± 0.01 <sup>b</sup>	0.17± 0.01 <sup>a</sup>	0.05± 0.01 <sup>b</sup>	0.04± 0.01 <sup>b</sup>	0.07± 0.02 <sup>b</sup>	0.12± 0.03 <sup>a</sup>	$0.27\pm 0.02^{a}$	0.07± 0.02 <sup>b</sup>	_d	-	-	-	-	-
C14:0	$0.26\pm 0.05^{c}$	$0.16\pm 0.02^{b}$	$\begin{array}{c} 0.53 \pm \\ 0.16^a \end{array}$	$\begin{array}{c} 0.47 \pm \\ 0.01^{b} \end{array}$	$\begin{array}{c} 0.22 \pm \\ 0.07^{b} \end{array}$	$\begin{array}{c} 0.38 \pm \\ 0.07^{ab} \end{array}$	$\begin{array}{c} 0.70 \pm \\ 0.17^a \end{array}$	$0.34\pm 0.09^{b}$	$0.30\pm 0.02^{b}$	$\begin{array}{c} 0.79 \pm \\ 0.05^a \end{array}$	$\begin{array}{c} 0.77 \pm \\ 0.16^a \end{array}$	$\begin{array}{c} 0.44 \pm \\ 0.14^a b \end{array}$	0.18± 0.05°	$\begin{array}{c} 0.26 \pm \\ 0.01^b \end{array}$	$\begin{array}{c} 0.27 \pm \\ 0.02^b \end{array}$
C14:1	$0.36 \pm 0.02^{b}$	0.35± 0.03 <sup>b</sup>	$0.25\pm 0.02^{b}$	0.26± 0.02°	$0.22\pm 0.02^{c}$	$0.21\pm 0.02^{b}$	$0.11\pm 0.02^{d}$	$0.14\pm 0.01^{d}$	$0.60\pm 0.35^{a}$	$0.34\pm 0.03^{b}$	0.23± 0.04°	$0.24\pm 0.02^{b}$	$\begin{array}{c} 0.50 \pm \\ 0.02^a \end{array}$	$0.52\pm 0.01^{a}$	$\begin{array}{c} 0.47 \pm \\ 0.05^{ab} \end{array}$
C15:0	1.19± 0.11°	1.12± 0.25 <sup>bc</sup>	1.82± 0.56 <sup>a</sup>	1.95± 0.16 <sup>a</sup>	$2.46\pm 0.35^{a}$	$2.53\pm 0.76^{a}$	2.51± 0.22 <sup>b</sup>	$2.21\pm 0.36^{a}$	$2.34\pm 0.79^{a}$	$2.02\pm 0.18^{a}$	1.53± 0.03 <sup>b</sup>	$2.88\pm 0.52^{a}$	1.10± 0.03°	0.86± 0.01°	1.98± 0.13 <sup>a</sup>
C16:0	26.03± 0.03 <sup>b</sup>	19.57± 0.05°		21.64± 0.38°		23.17± 0.08 <sup>a</sup>	19.57± 0.11 <sup>e</sup>	18.55± 0.11 <sup>d</sup>		21.71± 0.14 <sup>d</sup>	16.63± 0.13 <sup>e</sup>	22.47± 0.06 <sup>b</sup>			
C16:1n-7	0.03 0.24± 0.03°	0.03 0.34± 0.02°	0.07 0.34± 0.03 <sup>d</sup>	$0.31\pm 0.02^{c}$	0.08 0.32± 0.02°	0.30± 0.02 <sup>d</sup>	0.34± 0.03°	$0.36\pm 0.02^{c}$	1.02± 0.32 <sup>b</sup>	0.14 0.52± 0.02 <sup>b</sup>	0.13 0.51± 0.18 <sup>b</sup>	$0.00$ $2.02\pm$ $0.24^{a}$	1.75± 0.24 <sup>a</sup>	1.94±	$2.04\pm 0.06^{a}$
C17:0	0.50±	0.40±	0.43±	0.42±	$0.44\pm$	$0.44\pm$	0.38±	0.38±	$0.41\pm$	$0.48\pm$	0.55±	0.52±	0.41±	0.03 <sup>a</sup> 0.40±	$0.37\pm$
C17:1	$0.02^{a}$ $0.56\pm$	0.03° 0.51±	0.02 <sup>bc</sup> 0.82±	0.03 <sup>b</sup> 0.82±	0.01 <sup>b</sup> 0.94±	0.01 <sup>b</sup> 0.93±	0.02 <sup>b</sup> 1.02±	0.02° 0.86±	0.03° 1.16±	0.02 <sup>a</sup> 0.66±	0.03 <sup>a</sup> 0.57±	0.01 <sup>a</sup> 0.92±	0.01 <sup>b</sup> 0.41±	0.02° 0.35±	$0.02^{d}$ $0.65\pm$
C18:0	0.04 <sup>d</sup> 11.04±	0.09 <sup>bc</sup> 13.59±	0.23 <sup>ab</sup> 10.00±		0.12 <sup>a</sup> 13.15±	0.28 <sup>ab</sup> 13.28±	0.07 <sup>b</sup> 13.56±	0.13 <sup>a</sup> 14.61±			0.01 <sup>b</sup> 15.97±	0.18 <sup>ab</sup> 11.42±	0.01 <sup>e</sup> 13.50±	0.02° 11.62±	0.03 <sup>b</sup> 13.48±
C18:1n-9	0.03° 15.84±				0.04 <sup>d</sup> 15.48±		0.13 <sup>a</sup> 15.91±	0.03 <sup>b</sup> 13.24±		0.02 <sup>b</sup> 14.80±				0.02 <sup>e</sup> 13.80±	
	0.02 <sup>b</sup> 1.63±	0.05 <sup>e</sup> 1.48±	0.03° 1.20±	0.53 <sup>b</sup> 1.52±	0.02 <sup>b</sup> 1.48±	0.78 <sup>b</sup> 1.12±	0.03 <sup>b</sup> 1.00±	0.16 <sup>d</sup> 1.41±	0.24° 0.65±	0.04° 0.86±	0.02 <sup>a</sup> 1.53±	$0.28^{a}$ $0.50\pm$	$0.02^{a}$ $0.65\pm$	0.02° 0.61±	$\begin{array}{c} 0.85^a \\ 0.99 \pm \end{array}$
C18:1n-7	0.03 <sup>a</sup>	0.06 <sup>ab</sup>	$0.04^{a}$	0.31 <sup>a</sup>	0.02 <sup>ab</sup>	0.27 <sup>a</sup>	0.03 <sup>b</sup>	$0.10^{a}$	$0.03^{b}$	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.04 <sup>b</sup>	$0.02^{c}$	0.01°	0.38 <sup>ab</sup>
C18:2n-6	$25.91\pm 0.07^{a}$	29.86± 0.11 <sup>a</sup>	$21.23\pm 0.07^{c}$	22.29± 0.28°	$23.58\pm 0.07^{c}$	$21.91 \pm 0.03^{b}c$	$0.02^{d}$	23.09± 0.01 <sup>d</sup>	$23.48\pm 0.58^{a}b$	$0.02^{b}$	$23.57 \pm 0.05^{\circ}$	$23.58\pm 0.29^{a}$	$20.21 \pm 0.07^{d}$	$23.84\pm 0.02^{b}$	$22.56\pm 0.99^{b}$
C18:3n-3	$\begin{array}{c} 0.27 \pm \\ 0.03^d \end{array}$	$0.37 \pm 0.02^{c}$	$0.35\pm 0.01^{c}$	$\begin{array}{c} 0.41 \pm \\ 0.02^b \end{array}$	$0.37 \pm 0.02^{c}$	$\begin{array}{c} 0.31 \pm \\ 0.02^d \end{array}$	$\begin{array}{c} 0.48 \pm \\ 0.02^c \end{array}$	$\begin{array}{c} 0.54 \pm \\ 0.01^{ab} \end{array}$	$\begin{array}{c} 0.56 \pm \\ 0.03^{b} \end{array}$	$\begin{array}{c} 0.78 \pm \\ 0.06^a \end{array}$	$\begin{array}{c} 0.83 \pm \\ 0.37^a \end{array}$	$\begin{array}{c} 0.85 \pm \\ 0.02^a \end{array}$	$\begin{array}{c} 0.37 \pm \\ 0.02^b \end{array}$	$0.50\pm 0.03^{c}$	$\begin{array}{c} 0.54 \pm \\ 0.01^{b} \end{array}$
C20:0	$\begin{array}{c} 0.05 \pm \\ 0.01^a \end{array}$	$\begin{array}{c} 0.05 \pm \\ 0.01^b \end{array}$	$\begin{array}{c} 0.13 \pm \\ 0.03^a \end{array}$	$\begin{array}{c} 0.08 \pm \\ 0.03^a \end{array}$	$\begin{array}{c} 0.06 \pm \\ 0.01^b \end{array}$	$0.07 \pm 0.01^{b}$	$\begin{array}{c} 0.11 \pm \\ 0.03^a \end{array}$	$\begin{array}{c} 0.13 \pm \\ 0.03^a \end{array}$	$\begin{array}{c} 0.11 \pm \\ 0.01^{ab} \end{array}$	$\begin{array}{c} 0.09 \pm \\ 0.02^a \end{array}$	$\begin{array}{c} 0.09 \pm \\ 0.02^b \end{array}$	$0.09\pm 0.01^{b}$	$0.13\pm 0.02^{a}$	$\begin{array}{c} 0.08 \pm \\ 0.01^{b} \end{array}$	$\begin{array}{c} 0.07 \pm \\ 0.01^{b} \end{array}$
C20:1n-9	$\begin{array}{c} 0.18 \pm \\ 0.01^{ab} \end{array}$	$0.18\pm 0.02^{b}$	$0.26 \pm 0.01^{b}$	$0.11\pm 0.03^{b}$	$0.17 \pm 0.02^{b}$	$0.17 \pm 0.02^{b}$	$\begin{array}{c} 0.18 \pm \\ 0.06^{ab} \end{array}$	$\begin{array}{c} 0.27 \pm \\ 0.02^a \end{array}$	$\begin{array}{c} 0.30 \pm \\ 0.02^{ab} \end{array}$	$\begin{array}{c} 0.37 \pm \\ 0.03^a \end{array}$	$\begin{array}{c} 0.28 \pm \\ 0.03^a \end{array}$	$0.22 \pm 0.02^{b}$	$\begin{array}{c} 0.16 \pm \\ 0.02^{ab} \end{array}$	$\begin{array}{c} 0.27 \pm \\ 0.01^a \end{array}$	$\begin{array}{c} 0.37 \pm \\ 0.12^a \end{array}$
C20:2n-6	$\begin{array}{c} 0.68 \pm \\ 0.03^{d} \end{array}$	0.83± 0.02 <sup>e</sup>	$0.96 \pm 0.02^{c}$	$\begin{array}{c} 0.71 \pm \\ 0.03^{d} \end{array}$	$\begin{array}{c} 0.87 \pm \\ 0.02^d \end{array}$	$\begin{array}{c} 0.81 \pm \\ 0.03^{d} \end{array}$	1.19± 0.02°	$1.61\pm 0.02^{a}$	1.14± 0.03 <sup>b</sup>	1.65± 0.03 <sup>a</sup>	1.15± 0.01°	$\begin{array}{c} 1.23 \pm \\ 0.07^a \end{array}$	$0.99\pm 0.03^{b}$	1.29± 0.02 <sup>b</sup>	1.08± 0.05 <sup>b</sup>
C20:3n-6	$0.63\pm 0.02^{d}$	$0.83\pm 0.02^{d}$	$0.95 \pm 0.01^{d}$	0.91± 0.02 <sup>b</sup>	0.93± 0.02°	1.00± 0.03°	1.26± 0.03°	$1.38\pm 0.04^{a}$	1.28± 0.03 <sup>b</sup>	$1.54\pm 0.02^{a}$	1.18± 0.02 <sup>b</sup>	$1.38\pm 0.07^{a}$	$0.88 \pm 0.03^{b}$	$0.95\pm 0.02^{c}$	0.99± 0.05 <sup>cb</sup>
C20:4n-6					10.95± 0.18°						11.75± 0.05 <sup>b</sup>		8.81± 0.05°	9.84± 0.02 <sup>d</sup>	8.67± 0.41°
C20:5n-3	3.01± 0.04 <sup>d</sup>	3.77± 0.06°	$5.65\pm 0.06^{a}$	3.84± 0.02 <sup>b</sup>	3.58± 0.03 <sup>d</sup>	$3.73\pm 0.02^{c}$	4.60± 0.04°	4.34± 0.03 <sup>a</sup>	4.44± 0.15 <sup>b</sup>	4.21± 0.03 <sup>a</sup>	4.12± 0.01 <sup>b</sup>	$3.48\pm 0.10^{c}$	$2.77\pm 0.10^{e}$	3.09± 0.02 <sup>e</sup>	2.88± 0.14 <sup>d</sup>
C22:5n-3	1.08± 0.02e	1.40± 0.04 <sup>d</sup>	2.24± 0.03 <sup>a</sup>	1.67± 0.01 <sup>b</sup>	1.54± 0.02°	1.62± 0.02°	2.34± 0.02 <sup>ac</sup>	$2.33\pm 0.02^{a}$	$2.26\pm 0.07^{a}$	2.34± 0.01 <sup>a</sup>	1.87± 0.02 <sup>b</sup>	1.87± 0.08 <sup>b</sup>	1.12± 0.03°	1.34± 0.04 <sup>e</sup>	1.08± 0.01 <sup>d</sup>
C22:6n-3	$0.65\pm 0.02^{d}$	1.02± 0.15 <sup>b</sup>	1.60± 0.14 <sup>b</sup>	1.06± 0.02°	0.95± 0.03 <sup>bc</sup>	1.05± 0.01°	1.60± 0.03 <sup>b</sup>	1.66± 0.03 <sup>a</sup>	$1.87\pm 0.08^{a}$	1.29± 0.02°	1.05± 0.01 <sup>b</sup>	1.08± 0.04°	$0.69\pm 0.02^{d}$	$0.83\pm 0.02^{\circ}$	$0.76\pm 0.01^{d}$
SFAc					38.62± 0.56 <sup>b</sup>										40.03±
PUFA	42.04±	50.42±	48.91±	43.66±	42.77±	41.14±	44.48±	44.25±	45.75±	45.06±	45.51±	41.59±	35.81±	41.67±	
MUFA					$0.26^{d}$ $18.61\pm$ $0.09^{b}$			0.07 <sup>c</sup> 16.28± 0.14 <sup>d</sup>	$0.21^{b}$ $17.41\pm$ $0.48^{d}$	$0.07^{a}$ $17.55\pm$ $0.19^{d}$		0.82° 20.60± 0.49 <sup>b</sup>			
	0.07 <sup>c</sup>	0.16 <sup>e</sup>	0.16 <sup>e</sup>	0.77 <sup>b</sup>	0.09	0.77°	0.18 <sup>c</sup>	0.14	0.48	0.19	0.13ª	0.49	0.22ª	0.08°	1.28ª

<sup>&</sup>lt;sup>a</sup>Results were expressed as means±PE, data were means of three replicates.

<sup>&</sup>lt;sup>b</sup>Values of LD, LL and AM in the same row with different letters were significantly different, respectively.

 $<sup>^{</sup>c}$ SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids.  $^{d}$ ": undetected.

<sup>&</sup>lt;sup>e</sup>LD, *Longissimus dorsi*; LL, left-hind leg muscle; AM, abdominal muscle.

bits, the range of n-6/n-3 ratios in LD, LL, and AM were 7.34 to 8.47, 7.16 to 8.47, and 7.04 to 9.19, respectively. According to the FAO/WHO, the recommended intake of essential PUFA is 5/1 to 10/1 (n-6/n-3). Compared with the n-6/n-3 ratio of lean meats, rabbit loin possesses a fairly lower ratio than that of beef (8.9), pork loin (21.9), and chicken breast (15.8) and is comparable to that of veal loin meat (6.6) (Dalle Zotte and Szendrő, 2011).

# Effect of age and muscle on fatty acid composition of intramuscular phospholipids

The comparative intramuscular phospholipids in LD, LL, and AM of male Ira rabbit meat at different ages were shown in Table 3. The percentage of SFA and MUFA in LD, LL, and AM increased significantly (p<0.05) with increasing age of rabbits, whereas the percentage of PUFA among the muscles was significantly decreased (p <0.05). In addition, as shown in Fig. 4, during the growth of Ira rabbits, a significant reduction of PUFA/SFA ratio (A) and a significant increase in SFA + MUFA (B) were observed (p<0.05). High levels of UFA (the sum of PUFA and MUFA) (C), especially the abundance of PUFA, including the long chain (C20-22) PUFA in muscle (D), were observed in all samples. The PUFA proportion of the phospholipids in LL was significantly higher than that in total intramuscular lipid, indicating that the phospholipids contributed more PUFA in the total lipid rather than in the total triglycerides.

In terms of Ira rabbit meat at different ages, the SFAs among the muscles are mainly composed of C16:0 and C18:0. MUFA is mainly represented by C18:1, whereas PUFA consists of C18:2 and C20:4. These results agree with the results of the total intramuscular lipid composition of Ira rabbits. As the major ingredient of feeds for quite a lot species, the incorporation of C18:2n-6 into the muscles, in relation to the amount in the diet, is greatest among other fatty acids. C18:2n-6 is deposited in muscle phospholipids at a high level. Along with its long chain products (e.g., arachidonic acid (C20:4n-6)), C18:2n-6 incorporates well for insertion into phospholipids molecules (Wood *et al.*, 2008).

Long chain n-3 and n-6 PUFA are mainly found in phospholipid (Cooper *et al.*, 2004; Enser *et al.*, 2000). However, the fatty acids composition is seldom detected in different rabbit muscles during different feeding days. By comparing the variety of intramuscular phospholipids from LD, LL, and AM, we determined that C16:0, C14: 1n-6, C16:1n-7, C18:1n-9, C18:3n-3, C20:2n-6, and C20: 3n-6 were increased significantly (p<0.05). Both C20:4n-

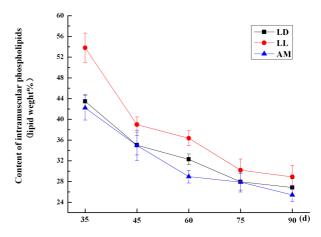


Fig. 4. The ratio of PUFA/SFA, content of SFA+MUFA, UFA, PUFA, and C20-22 PUFA of intramuscular phospholipids in male Ira rabbits at different ages and muscles. (LD, Longissimus dorsi; LL, left-hind leg muscle; and AM, abdominal muscle)

6 and C20:5n-3 decreased significantly (p<0.05). No significant differences were observed in C15:0 and C17:1. In terms of C18:0, LL was sequentially higher than LD and AM. For the C18:1n-9 proportion, LD was the highest, whereas AM was the lowest. These results were consistent with the characteristics of the obtained intramuscular lipid composition. The content of C18:2n-6 in LL was the highest. No significant variation was found among other fatty acids components. During the growth of Ira rabbits, the values of n-6/n-3 values in LD, LL, and AM was 3.93 to 7.38, 4.32 to 6.67, and 3.97 to 6.33, respectively. According to FAO/WHO, the recommended ratio of essential PUFA in a healthy daily diet was 5/1 to 10/1 (n-6/n-3). Nutritional value is determined primarily by the ratio between SFA and PUFA in meat and the balance between fatty acids of the n-6 and n-3 series. In general, a ratio of PUFA to SFA (termed P:S) above about 0.45 and a ratio of n-6:n-3 below about 4.0 are required in the diet tocombat various "lifestyle diseases" such as coronary heart disease and cancers (Simopoulos, 2004; Williams, 2000). Besides, a lower ratio is more desirable in reducing the risk of many chronic diseases. The optimal ratio varies depending on the disease under consideration (Simopoulos, 2002). Therefore, Ira rabbits may have excellent fatty acid profile and have potential nutritive value.

### **Conclusions**

Compared with other animal meats, Ira rabbits are a meat source of excellent quality and contain low fat, low n-6/n-3 ratio, high phospholipids, and PUFA. As for the

total intramuscular fatty acids, SFA consists of C16:0 and C18:0; MUFA consists of C18:1; and PUFA consists of C18:2 and C20:4. The content of total intramuscular lipid increases with age, whereas the phospholipids content (total intramuscular lipid weight%) decreases with the progressing age. However, the absolute percentage of intramuscular phospholipids in muscles does not significantly vary. During the 35 d to 90 d growth period, significant differences existed in the fatty acid composition of total intramuscular lipid and phospholipids in the LD, LL, and AM of male Ira rabbits. Considering these differences, the nutritional characteristic of the different muscles of Ira rabbits is a promising research topic. Further investigation is necessary to explore the interaction between meat quality and fatty acids composition.

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#### References

- 1. Bartlett, G. R. (1959) Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**, 466-468.
- 2. Bianchi, M., Petracci, M., and Cavani, C. (2009) The influence of linseed on rabbit meat quality. *World Rabbit Sci.* 17, 97-107.
- 3. Cappelli, P. and Vannucchi, V. (2005) Trasformazione delle carni: i salumi. In Chimica degli alimenti. Bologna, Italy: Zanichelli editore, pp. 500.
- 4. Cavani, C., Petracci, M., Trocino, A., and Xiccato, G. (2009) Advances in research on poultry and rabbit meat quality. *Italian J. Anim. Sci. (Supplement)* **8**, S741-S750.
- 5. Combes, S. and Dalle Zotte, A. (2005) Rabbit meat: Dietetic properties and processing characteristics. 11émes Journées de la Recherche Cunicole, Paris (France), pp. 167-180.
- Conquer, J. A. and Holub, B. J. (1998) Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esteried fatty acid in subjects of Asian Indian background. *J. Lipid Res.* 39, 286-292.
- Cooper, S. L., Sinclair, L. A., Wilkinson, R. G., Hallett, K. G., Enser, M., and Wood, J. D. (2004) Manipulation of the n-3 polyunsaturated acid content of muscle and adipose tissue in lambs. *J. Anim. Sci.* 82, 1461-1470.
- 8. Dalle Zotte, A. and Szendrő, Z. (2011) The role of rabbit meat as functional food. *Meat Sci.* **88**, 319-331.

- 9. Dalle Zotte, A. (2002) Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livestock Prod. Sci.* **75**, 11-32.
- Dalle Zotte, A. and Szendró', Z. (2010) The role of rabbit meat as functional food. CD of 4th Cuniculture Congress of the Americas, Córdoba, Argentina.
- 11. Enser, M., Richardson, R. I., Wood, J. D., Gill, B. P., and Sheard, P. R. (2000) Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sausages. *Meat Sci.* **55**, 201-212.
- 12. FAOSTAT (2010) http://faostat.fao.org/site/291/default.aspx
- 13. Folch, J., Lees, M., and Stanley, G. H. S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**, 497-509.
- 14. Gigaud, V. and Combes, S., (2008) The effect of decreasing the omega 6/omega 3 ratio in feed on fatty acid content of rabbit meat to meet human dietary recommendations. 9th World Rabbit Congress, Verona, Italy, pp. 1353-1357.
- 15. Gray, J. I., Gomaa, E. A., and Buckley, D. J. (1996) Oxidative quality and shelf life of meats. *Meat Sci.* **43**, S111-S123.
- Hernàndez, P. and Gondret, F. (2006) Rabbit meat quality. In Maertens, L. and Coudert, P. (Eds.), Recent advances in rabbit sciences, Melle, Belgium: ILVO. pp. 269-290.
- 17. Hernández, P. (2008) Enhancement of nutritional quality and safety in rabbit meat. 9th World Rabbit Congress, Verona, Italy, pp. 367-383.
- Hernàndez, P. and Dalle Zotte, A. (2010) Influence of diet on rabbit meat quality. (pp 163-178). In: Nutrition of the rabbit. Edited by C. de Blas, Universidad Poletenica, Madrid, J. Wiseman, University of Nottingham, UK, 2nd ed., ISBN-13: 9781845936693.
- Hernández, P. and Gondret, F. (2006) Rabbit meat quality. In: Maertens, L., Coudert, P. (Eds.), Recent advances in rabbit sciences. ILVO, Belgium, pp. 269-290.
- 20. Juaneda, P. and Rocquelin, G. (1985) Rapid and convenient separation of phospholipids and nonphosphorus lipids from rat heart using silica cartridges. *Lipids* **20**, 40-41.
- 21. Kouba M. and Biochimie J. M. (2011) A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids, *Biochimie* **93**, 13-17.
- Littell, R. C., Milliken, G. A., Stroup, W. W., and Wolfinger, R. D. (1996) SAS System for Mixed Models. SAS Institute, Incorporated, pp. 633.
- Maertens, L., Huyghebaert, G., and Delezie, E. (2008) Fatty acid composition of rabbit meat when fed a linseed based diet during different periods after weaning. 9th World Rabbit Congress, Verona, Italy, pp. 1381-1385.
- 24. Molette, C., Nicot, M. C., Coulmier, D., Farizon, Y., and Gidenne, T. (2009) High incorporation of distillers grains of wheat in a simplified feed formulation. Impact on growth, carcass quality and fatty acid composition of the rabbit meat. 13émes Journées de la Recherche Cunicole, LeMans, France, pp. 14-17.
- 25. Morrison, W. R. and Smith, L. M. (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron

- fluoride-methanol. J. Lipid Res. 5, 600-608.
- 26. NRC [National Research Council] (1985) Guide for the Care and Use of Laboratory Animals, sixth ed. National Academy Press, Washington, D.C.
- 27. Petracci, M., Bianchi, M., and Cavani, C. (2009) Development of rabbit meat products fortified with n-3 polyunsaturated fatty acids. *Nutrients* **1**, 111-118.
- Raes, K., De Smet, S., and Demeyer, D. (2004) Effect of dietary fatty acids onincorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. *Anim. Feed Sci. Technol.* 113, 199-221.
- Salma, U., Miah, A. G., Maki, T., Nishimura, M., and Tsujii, H. (2007) Effect of dietary *Rhodobacter capsulatus* on cholesterol concentration and fatty acid composition in broiler meat. *Poult. Sci.* 86, 1920-1926.

- 30. Simopoulos, A. P. (2001) N-3 fatty acids and human health: defining strategies for public policy. *Lipids (Supplement)* **36**, S83-S89.
- 31. Simopoulos, A. P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* **56**, 365-379.
- Simopoulos, A. P. (2004) Omega-3 fatty acids and antioxidants in edible wild plants. *Biol. Res.* 37, 263-277.
- 33. Williams, C. M. (2000) Dietary fatty acids and human health. *Annales de Zootechnie* **49**,165-180.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., and Whittington, F. M. (2008) Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.* 78, 343-358.

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