PAPER

COMPOSITION OF INTRAMUSCULAR PHOSPHOLIPID FATTY ACIDS OF INRA RABBIT AT DIFFERENT AGES

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ABSTRACT

The composition of intramuscular phospholipids fatty acids in *Longissimus dorsimuscle* (LD), left-hind leg muscle (LL) and abdominal muscle (AM) of Inra rabbit slaughtered between 35 to 90 days old were investigated. Significant decreasing of intramuscular phospholipids (% total intramuscular lipids) was observed in three muscles as age increased ($p < 0.05$). The highest phospholipids content was found in LL in both male and female rabbits during the growth period, and the phospholipids content in three muscles of the males were higher than that of the females. Abundant amount of unsaturated fatty acids (UFA), especially polyunsaturated fatty acids (PUFA) characterised the fatty acid composition of the intramuscular phospholipids (32.94-55.79%), and the percentage of PUFA in the muscles were all significantly decreased during the growth of male and female Inra rabbit ($p < 0.05$). In addition, a significant reduction of PUFA/SFA ratio and a significant increase of SFA + MUFA were observed ($p < 0.05$). Major fatty acids, such as Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6) and arachidonic acid (C20:4) changed more obviously than other fatty acids. The analysis of partial least square regression (PLSR) showed that the composition of phospholipid fatty acids varied in age, muscle and gender, and the nutritional value of the phospholipid fatty acids decreased with age distinctly, the AM had better nutritional value of phospholipids.

Keywords: rabbit, fatty acids, intramuscular phospholipids, composition

1. INTRODUCTION

Functional foods are a tool that can be easily used in reducing public health costs. Compared to meats of other animal species, rabbit meat is characterized by high levels of polyunsaturated fatty acids (PUFA) and n-3 fatty acids, high levels of protein with essential amino acids, high digestibility value, lower cholesterol contents and significant source of vitamin B family (vitamins B_2, B_5, B_6, B_3, B_{12}) etc. (DALLE ZOTTE and SZENDRÖ, 2011). Moreover, rabbit meat consumption could become a good way of providing bioactive compounds to human consumers, since the rabbit meat fatty acids profile may be favorably modified by the inclusion of raw materials rich in unsaturated fatty acids (UFA) in the diet (DAL BOSCO *et al*., 2004; HERNÁNDEZ, 2008; KOUBA *et al*., 2008). Rabbit meat is considered as dietetically healthy, relatively rich in n-3 PUFAs and with a lower n-6 to n-3 ratio (7～12) than pork, veal or chicken meats (DALLE ZOTTE, 2002; HERNÁNDEZ and GONDRET, 2006). Unlike pork or beef meat, it contains two important metabolites from α-linolenic acid (ALA), docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) in detectable levels (COMBES and DALLE ZOTTE, 2005; EIBEN *et al*., 2010). Inra rabbit is imported from France and have high breeding efficiency. In recent years, interests have focused not only on the amount of intramuscular phospholipids but also on the composition of fatty acids. The intramuscular fat (IMF), which characterises the amount of fat, is one of the major factors affecting the palatability of meat (HOCQUETTE *et al*., 2010). Muscle lipids are composed of polar lipids, mainly phospholipids (rich in PUFA) located in the cell membranes, and triacylglycerols (high levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA)) along the muscle fibres (DE SMET *et al*., 2004). In the IMF, PUFA are restricted almost exclusively to the phospholipids fraction (WOOD *et al*., 2003). Thus, the amount of intramuscular phospholipids in the meat is an important factor (GRAY *et al*., 1996). Phospholipids consist of long-chain fatty acids attached to a phosphoryl group. Since the fatty acids chains can vary in length and degree of saturation, each phospholipid class possesses numerous molecular species with different chemical and biological properties (MARCO *et al*., 2004; WANG *et al*., 2009).

CAMBERO *et al*. (1991) first analysed the phospholipid content and classes in rabbits and they provide important information on phospholipid prevalence according to breed and feeding, specifying that phospholipid differs also according to age and gender. Generally, the analysis of phospholipids is based the determination of the phospholipid classes (phosphatidyleth-anolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, sphingomyelin, lysophosphatidylcholine etc.) with high performance liquid chromatography (ALASNIER and GANDEMER, 1998; PETERSON and CUMMINGS, 2005; BOSELLI *et al*., 2008). However, practically limited literature data are available on the determination of the fatty acids in intramuscular phospholipids by gas chromatography after purification of the polar lipid fraction, especially the intramuscular phospholipids from Inra rabbit. Hence, in order to provide a database for the characterization of nutritional quality of Inra rabbit meat, the composition of intramuscular phospholipids fatty acids and the effect of ages, genders and muscles on the composition and nutritional value of fatty acids were investigated by gas chromatography.

2. MATERIALS AND METHODS

2.1. Sampling of Inra rabbit meat

A total of 200 35 days old weaned Inra rabbits (20 males+20 females 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.

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[a]	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[b]	10.5 MJ/kg

[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.
[b] Digestible energy (kcal/kg DM) = TDN×4400 (NRC, 1985).

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 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. The facilities of the slaughterhouse met the requirements of the Institute of Animal Care and Use Committee (IACUC), which is funded by the United States National Institutes of Health. After 24 h post-mortem, the longissimus dorsi muscle (LD), left-hind leg muscle (LL), and abdominal muscle (AM) (ventral musculus) of the carcass were removed and immediately vacuum-packed and frozen at -20°C until analyzed.

2.2. Intramuscular lipid content and fatty acid composition analysis

Intramuscular lipids were extracted according to FOLCH *et al*. (1957). Total lipid content was measured by weighing after solvent evaporation. The content of IMF was expressed as percent of the muscle weight. Fractions of intramuscular phospholipids were prepared with silica cartridges (Sep-Pack, Waters, Milford, MA, USA) by the method of JUANEDA and ROCQUELIN (1985). Phospholipids were quantified by phosphorous determination (BARTLETT, 1959). The relative content of phospholpids was expressed as percent of the IMF weight, while the absolute content was expressed as percent of the muscle weight. The phospholipids were methylated with boron fluoride-methanol (Sigma Aldrich) according to MORRISON and SMITH (1964). The fatty acids methyl esters were analyzed by a QP-2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a split injector. One microliter of FA methyl esters was injected in split mode (5:1) onto a Rtx-Wax capillary column (Restek, Bellefonte, PA, USA; 30 m × 0.25 mm id × 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 then increments of 5°C /min to 230°C and held at 230°C for 10 min. The temperature of the injector and the detector both were 250°C. The flow rate of the carrier gas (N_2) was 1.5 mL/min. Identification of fatty acids was performed by comparison of the retention times with those of standards (Sigma). The results were expressed as percent of the total fatty acids methyl esters present.

2.3. Statistical analysis

The Statistical Analysis System (1996) was used to determine means, standard errors and analysis of variance. Duncan's multiple range test was used to compare differences among means. An alpha level of $p < 0.05$ was considered significant.
The effect of ages, muscles and genders on the composition of intramuscular phospholipid fatty acids were performed by ANOVA-partial least squares regression (A-PLSR). Ten 0/1 indicators variables (35, 45, 60, 75, 90 d, female, male, LD, LL, AM), SFA+MUFA, and PUFA/SFA in the X-matrix and 21 kinds of fatty acids (C12:0 - C22: 6n-3 were represented by the number 1-21) in the Y-matrix. Ellipses represent R^2=0.5 (50%) and 1.0 (100%). A PLSR was performed using the Unscrambler Software, version 9.7 (CAMO ASA, Trondheim, Norway). All data was centered and standardized before analysis.

3. RESULTS AND DISCUSSIONS

3.1. Variation of content of total intramuscular lipids and phospholipids of Inra rabbits

3.1.1. Variation of intramuscular lipid content

The intramuscular lipid content (% muscle weight) of LD, LL and AM from male and female Inra rabbits were significantly increased (p <0.05) with age (Table 2). The intramuscular lipid content of the three muscles of male and female rabbits was all increased. During the growth of Inra rabbits, the AM showed the highest content of intramuscular lipid, followed by LL and LD. HERNÀNDEZ and DALLE ZOTTE (2010) reported that the leanest cut of meat in the rabbit carcass was the loin and the hindleg was the quantitatively important cut because of its low lipid content compared to the other meats, which was consistent with our investigation (male: 0.77-1.21%, female: 0.79-1.33%).

Hence, the lipid content depended greatly on the age, gender and muscle. During the growth period from 35 d to 90 d, the deposition degree of total intramuscular lipid of the females in AM (2.88% to 5.42%) was significantly higher than that in the LL (1.19% to 1.78%) and LD (0.79% to 1.33%).

Table 2: Comparison of intramuscular lipid content of Inra rabbit at different ages[a].

	LD[a]		LL		AM	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
35 d[bc]	0.77±0.02d	0.79±0.08C	1.16±0.12d	1.19±0.15C	2.41±0.16d	2.88±0.12E
45 d	0.80±0.08cd	0.82±0.06C	1.20±0.06d	1.24±0.12C	2.67±0.09c	3.18±0.08D
60 d	0.96±0.11bc	1.01±0.10BC	1.30±0.03c	1.45±0.13B	2.74±0.01c	3.58±0.20C
75 d	1.15±0.13ab	1.25±0.24AB	1.49±0.12b	1.64±0.07AB	4.60±0.03b	4.83±0.05B
90 d	1.21±0.13a	1.33±0.21A	1.66±0.02a	1.78±0.06A	5.16±0.20a	5.42±0.17A

[a] LD, Longissimus dorsimuscle; LL, left-hind leg muscle; AM, abdominal muscle.
[b] Results were expressed as means ± SE, data were means of three replicates.
[c] Values in the same column with different letters were significantly different ($p<0.05$), male: a-d, female: A-E.

3.1.2. Variation of intramuscular phospholipids content

The percentage of phospholipids in the total intramuscular lipids of both male and female Inra rabbits was significantly decreased at the three muscles with age ($p<0.05$) (Table 3). A higher percentage of phospholipids characterized the total lipids (22.35-53.81%), however, the phospholipid contents in the meat of both New Zealand white and the commercial hybrid ranged from 9% to 19% total lipid (CAMBERO *et al*., 1991).

Table 3: Comparison of intramuscular phospholipids content (intramuscular lipid weight %) of Inra rabbit at different ages[a].

	LD[a]		LL		AM	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
35 d[bc]	43.47±1.33a	42.11±1.96A	53.81±2.82a	51.03±2.22A	42.21±2.36a	37.01±1.98A
45 d	35.02±1.88b	33.64±1.53B	38.98±1.48b	36.78±1.65B	34.94±2.88b	31.99±1.68B
60 d	32.28±1.02b	31.14±1.92B	36.35±1.44b	33.87±1.60B	28.93±1.22c	25.31±1.16C
75 d	27.93±1.58c	24.13±0.17C	30.21±2.14c	27.84±2.39C	27.87±1.91c	23.36±2.96C
90 d	26.84±1.72c	23.52±0.86C	28.89±2.18c	26.78±1.16C	25.40±1.32c	22.35±1.29C

[a] LD, Longissimus dorsimuscle; LL, left-hind leg muscle; AM, abdominal muscle.
[b] Results were expressed as means ± SE, data were means of three replicates.
[c] Values in the same column with different letters were significantly different ($p<0.05$), male: a-c, female: A-C.

The highest relative phospholipids content was found in the LL in both male and female Inra rabbits at different ages. In addition, the relative content of phospholipids in Inra rabbit abdomen showed significant gender differences, whereas that in legs and back phospholipids content appeared more pronounced gender differences after the age of 60 d. However, the absolute percentage of intramuscular phospholipids in the three muscles

did not vary obviously ($p > 0.05$) (Fig. 1). Among the three muscles, the AM showed the maximum absolute percentage of intramuscular phospholipids, followed by LL and LD, and the absolute percentage of intramuscular phospholipids in males were higher than that in females. According to wood *et al* (2008), phospholipids remained constant or increase little, as muscle fatness increases, whilst triacylglycerols increased to a higher extent, which may explain this phenomenon.

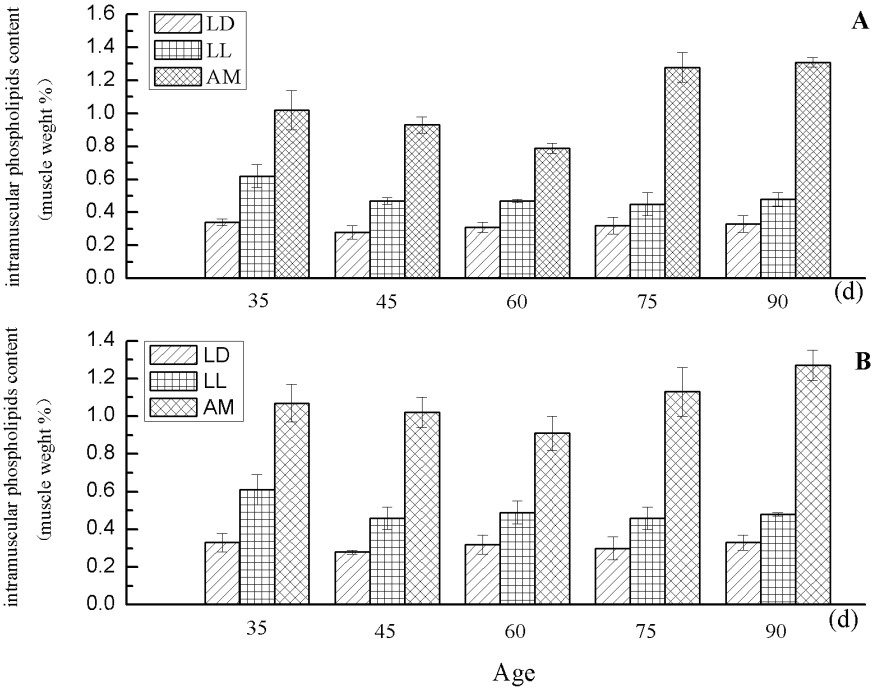

Figure 1: Comparison of intramuscular phospholipids content (muscle weight %) of Inra rabbit at different ages (A: male rabbits, B: female rabbits).

3.2. Effect of age, muscle and gender on composition of intramuscular phospholipids

The comparative intramuscular phospholipids fatty acids in LD, LL and AM of both male and female Inra rabbits at 35, 45, 60, 75, 90 d were shown in Table 4 (LD), Table 5 (LL), and Table 6 (AM), respectively. High levels of UFA (the sum of PUFA and MUFA), especially the abundance of PUFA, including the long chain (C20-22) PUFA in muscle, were observed in all samples. In muscle, significant percentage is phospholipids, which has a much higher PUFA content in order to perform its function as a constituent of cellular membranes (Wood *et al*., 2008). The ratio of SFA and MUFA in LD, LL and AM increased significantly with age in both gender rabbits ($p < 0.05$), whereas the ratio of PUFA among the muscles were all significantly decreased ($p < 0.05$). In addition, a significant reduction of PUFA/SFA ratio and a significant increase of SFA + MUFA were observed ($p < 0.05$).
During the growth, the phospholipids PUFA percentage (% intramuscular fatty acids) was significantly higher in the LL than that in the LD and AM of male rabbits, corresponding to the lowest MUFA percentage in male LL. The phospholipids PUFA percentage in female LD was the lowest, while the MUFA in female legs was the minimum. Compared to the LD and LL, the AM existed more obvious gender differences in SFA and UFA percentage of phospholipids.

Table 4: Composition of the fatty acids of intramuscular phospholipids (%) in Longissimus dorsi of Inra rabbit at different ages[a].

	35d		45d		60d		75d		90d	
	male	female	male	female	male	female	male	female	male	female
C12:0[b]	0.10±0.02a	0.03±0.01C	0.05±0.01b	0.08±0.02B	0.12±0.03a	0.17±0.02A	-[d]	-	-	-
C14:0	0.26±0.05e	0.28±0.05B	0.47±0.01d	0.52±0.15A	0.60±0.17c	0.57±0.07A	0.79±0.05b	0.58±0.05A	0.83±0.05a	0.53±0.04A
C14:1	0.36±0.02b	0.41±0.02A	0.26±0.02c	0.46±0.04A	0.11±0.02d	0.41±0.06A	0.34±0.03b	0.46±0.11A	0.50±0.02a	0.46±0.02A
C15:0	1.19±0.11d	1.46±0.22E	1.65±0.16c	1.57±0.10D	1.81±0.22b	2.47±0.31C	2.02±0.18a	3.20±0.32A	1.10±0.03d	2.79±0.13B
C16:0	20.03±0.03e	17.16±0.19E	21.64±0.38d	20.30±0.05D	22.37±0.11c	21.25±0.09C	23.71±0.14b	23.34±0.12B	25.11±0.30a	28.22±0.02A
C16:1n-7	0.24±0.03c	0.29±0.03D	0.31±0.02c	0.48±0.06A	0.34±0.03c	0.39±0.02C	0.52±0.02b	0.44±0.04B	1.75±0.24a	0.44±0.01B
C17:0	0.50±0.02a	0.48±0.02A	0.42±0.03b	0.47±0.04A	0.38±0.02b	0.39±0.02B	0.48±0.02a	0.40±0.01B	0.41±0.01b	0.50±0.02A
C17:1	0.56±0.04d	0.72±0.09E	0.82±0.05a	1.34±0.07A	0.92±0.07b	1.15±0.07B	0.66±0.05c	0.93±0.05D	0.41±0.01e	1.01±0.03C
C18:0	11.04±0.03e	12.58±0.40D	11.72±0.07d	13.75±0.10C	12.56±0.13c	13.82±0.03C	13.11±0.02b	14.31±0.24B	13.50±0.03a	14.45±0.04A
C18:1n-9	13.34±0.02e	13.54±0.15E	13.99±0.53d	13.69±0.15D	15.01±0.03c	14.43±0.03C	15.80±0.04b	14.61±0.06B	17.89±0.02a	17.75±0.07A
C18:1n-7	1.63±0.03a	1.64±0.21A	1.52±0.31a	0.88±0.12D	1.00±0.03b	1.06±0.01C	0.86±0.02b	1.15±0.02B	0.65±0.02c	0.65±0.02E
C18:2n-6	25.91±0.07a	25.00±0.23A	24.29±0.28b	20.24±0.13B	22.67±0.02c	19.14±0.10C	20.77±0.02d	17.65±0.11D	20.21±0.07e	15.09±0.08E
C18:3n-3	0.27±0.03d	0.35±0.03B	0.41±0.02b	0.58±0.02A	0.48±0.02c	0.58±0.03A	0.78±0.06a	0.56±0.02A	0.37±0.02b	0.38±0.12B
C20:0	0.05±0.01a	0.06±0.01B	0.08±0.03a	0.17±0.01A	0.11±0.03a	0.17±0.02A	0.09±0.02a	0.17±0.01A	0.13±0.02a	0.16±0.06A
C20:1n-9	0.18±0.01ab	0.17±0.02B	0.11±0.03b	0.11±0.03C	0.18±0.06ab	0.22±0.02A	0.37±0.03a	0.14±0.01BC	0.16±0.02ab	0.10±0.02C
C20:2n-6	0.68±0.03d	0.73±0.03E	0.71±0.03d	0.83±0.06D	1.19±0.02c	0.90±0.03C	1.65±0.03a	1.15±0.02B	1.74±0.03b	1.23±0.01A
C20:3n-6	0.63±0.02e	0.68±0.04C	0.91±0.02d	1.21±0.07A	1.26±0.03c	1.20±0.06A	1.54±0.02b	1.21±0.04A	1.88±0.03a	0.82±0.05B
C20:4n-6	13.80±0.08a	14.58±0.05A	11.78±0.08b	14.01±0.13B	9.72±0.14c	13.64±0.15C	9.47±0.04d	12.99±0.11D	8.81±0.05e	10.36±0.10E
C20:5n-3	5.01±0.04a	5.53±0.03A	4.84±0.02b	5.03±0.12B	4.60±0.04c	4.60±0.03C	3.91±0.03d	4.04±0.06D	2.77±0.10e	3.40±0.02E
C22:5n-3	2.58±0.02a	2.49±0.03A	2.47±0.01b	2.33±0.08B	2.34±0.02c	2.21±0.04C	1.84±0.01d	1.62±0.04D	1.12±0.03e	1.11±0.03E
C22:6n-3	1.65±0.02a	1.83±0.02A	1.56±0.02b	1.41±0.04B	1.40±0.03c	1.23±0.01C	1.29±0.02d	1.03±0.01D	0.69±0.02e	0.56±0.03E
SFA[c]	33.16±0.12e	32.06±0.06E	36.03±0.69d	36.86±0.78D	37.96±0.11c	38.83±0.34C	40.19±0.13b	41.99±0.26B	41.07±0.31a	46.65±0.07A
PUFA	50.54±0.17a	51.18±0.24A	46.96±0.34b	45.64±0.07B	43.68±0.17c	43.51±0.35C	41.26±0.07d	40.27±0.30D	37.56±0.09e	32.94±0.09E
MUFA	16.30±0.07e	16.76±0.28D	17.02±0.77d	17.50±0.85C	18.36±0.18c	17.66±0.06BC	18.55±0.19b	17.74±0.04B	21.37±0.22a	20.41±0.02A

[a] Results were expressed as means ± SE, data were means of three replicates.
[b] Values in the same column with different letters were significantly different ($p<0.05$), male: a-e, female: A-E.
[c] SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids.
[d] "-" : undetected.

Table 5: Composition of the fatty acids of intramuscular phospholipids (%) in left-hind leg muscle of Inra rabbit at different ages[a].

	35d		45d		60d		75d		90d	
	male	female	male	female	male	female	male	female	male	female
C12:0[b]	0.05±0.01b	0.04±0.01B	0.04±0.01b	0.09±0.01A	0.27±0.02a	0.05±0.02B	-[d]	-	-	-
C14:0	0.16±0.02b	0.23±0.06D	0.22±0.07b	0.41±0.11C	0.34±0.09b	0.54±0.13BC	0.77±0.16a	0.95±0.11A	0.26±0.01b	0.68±0.02B
C14:1	0.35±0.03b	0.37±0.02B	0.22±0.02c	0.14±0.02C	0.14±0.01d	0.51±0.03A	0.23±0.04c	0.32±0.01B	0.52±0.01a	0.52±0.03A
C15:0	1.12±0.25d	1.55±0.09B	1.46±0.35c	1.60±0.09B	1.51±0.36bc	2.39±0.47A	1.53±0.03b	1.77±0.13B	1.66±0.01a	1.84±0.07B
C16:0	19.57±0.05e	16.98±0.13D	20.65±0.08d	20.48±0.07C	21.55±0.11c	21.95±0.06B	22.63±0.13b	22.01±0.22B	24.08±0.05a	24.66±0.20A
C16:1n-7	0.34±0.02c	0.37±0.02D	0.32±0.02c	0.39±0.02CD	0.36±0.02c	0.41±0.02C	0.51±0.18b	0.75±0.02A	1.94±0.03a	0.48±0.01B
C17:0	0.40±0.03c	0.43±0.01B	0.44±0.01b	0.36±0.01C	0.38±0.02c	0.50±0.02A	0.55±0.03a	0.40±0.03B	0.40±0.02c	0.49±0.01A
C17:1	0.51±0.09bc	0.67±0.02B	0.94±0.12a	2.08±0.29A	0.86±0.13a	0.89±0.11B	0.57±0.01b	0.54±0.05B	0.35±0.02c	0.78±0.02B
C18:0	11.79±0.06e	13.12±0.06E	13.15±0.04d	14.78±0.10D	14.61±0.03c	15.29±0.10C	15.97±0.04b	15.91±0.03B	16.12±0.02a	16.32±0.03A
C18:1n-9	11.78±0.05e	12.09±0.12E	12.48±0.02d	12.34±0.06D	13.24±0.16c	13.60±0.12C	14.83±0.02b	13.94±0.15B	15.10±0.02a	15.75±0.07A
C18:1n-7	1.48±0.06ab	1.39±0.03A	1.48±0.02ab	0.85±0.01C	1.41±0.10a	0.99±0.05B	1.53±0.02b	1.00±0.08B	0.61±0.01c	0.70±0.03D
C18:2n-6	29.86±0.11a	26.34±0.02A	26.58±0.07b	20.62±0.25B	24.59±0.01c	18.77±0.72C	21.37±0.05d	18.21±0.02D	20.84±0.02e	15.45±0.05E
C18:3n-3	0.37±0.02c	0.37±0.02CD	0.37±0.02c	0.72±0.06A	0.54±0.01ab	0.40±0.02C	0.83±0.37a	0.56±0.01B	0.50±0.03c	0.35±0.04D
C20:0	0.05±0.01b	0.07±0.01A	0.06±0.01b	0.17±0.03A	0.13±0.03a	0.17±0.18A	0.09±0.02b	0.12±0.03A	0.08±0.01b	0.13±0.01A
C20:1n-9	0.18±0.02b	0.18±0.02BC	0.17±0.02b	0.11±0.02D	0.27±0.02a	0.20±0.02B	0.28±0.03a	0.28±0.01A	0.27±0.01a	0.15±0.02C
C20:2n-6	0.83±0.02d	0.83±0.02E	0.87±0.02c	0.93±0.01D	1.11±0.02b	1.03±0.02C	1.15±0.01b	1.41±0.02B	1.29±0.02a	1.53±0.02A
C20:3n-6	0.83±0.02d	0.78±0.02E	0.93±0.02c	1.08±0.02D	1.08±0.04b	1.04±0.01C	1.18±0.02a	1.10±0.02B	0.95±0.02c	1.16±0.01A
C20:4n-6	12.33±0.10a	15.29±0.07A	11.95±0.18b	14.42±0.07B	10.29±0.02c	13.94±0.05C	9.95±0.05d	13.56±0.09C	9.84±0.02d	12.59±0.07D
C20:5n-3	3.77±0.06a	4.93±0.02A	3.58±0.03b	4.73±0.05B	3.34±0.03c	4.45±0.11C	3.12±0.01d	4.21±0.03D	3.09±0.02e	4.17±0.05D
C22:5n-3	2.40±0.04a	2.33±0.02A	2.34±0.02b	2.31±0.03B	2.33±0.02b	2.06±0.04C	1.87±0.02c	1.79±0.03D	1.34±0.04d	1.59±0.08E
C22:6n-3	1.82±0.15a	1.63±0.02A	1.75±0.03a	1.37±0.02B	1.66±0.03b	1.27±0.28C	1.05±0.01c	1.17±0.02D	0.83±0.02d	0.67±0.04E
SFA[c]	33.14±0.29e	32.44±0.06D	36.02±0.56d	37.90±0.01C	38.77±0.11c	40.89±0.33B	41.53±0.23b	41.16±0.04B	42.60±0.07a	44.13±0.11A
PUFA	52.22±0.45a	52.50±0.10A	48.37±0.26b	46.19±0.22B	41.95±0.07c	42.51±0.39C	40.51±0.35d	42.01±0.19D	38.67±0.03e	37.50±0.12E
MUFA	14.64±0.16e	15.07±0.14D	15.61±0.09d	15.91±0.21C	16.28±0.14c	16.61±0.07B	17.96±0.13b	16.83±0.19B	18.72±0.08a	18.37±0.08A

[a] Results were expressed as means ± SE, data were means of three replicates.
[b] Values in the same column with different letters were significantly different ($p<0.05$), male: a-e, female: A-E.
[c] SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids.
[d] "-" : undetected.

Table 6: Composition of the fatty acids of intramuscular phospholipids (%) in abdominal muscle of Inra rabbit at different ages[a].

	35d		**45d**		**60d**		**75d**		**90d**	
	male	female	male	female	male	female	male	female	male	female
C12:0[b]	0.17±0.01a	0.07±0.02A	0.07±0.02b	0.04±0.01B	0.07±0.02b	0.06±0.01AB	-[d]	-	-	-
C14:0	0.53±0.16a	0.39±0.05D	0.38±0.07ab	0.65±0.19A	0.30±0.02b	0.41±0.06C	0.44±0.14ab	0.59±0.04B	0.27±0.02b	0.69±0.03A
C14:1	0.25±0.02b	0.15±0.02D	0.21±0.02b	0.18±0.01C	0.60±0.35a	0.27±0.01B	0.24±0.02b	0.28±0.02B	0.47±0.05ab	0.56±0.19A
C15:0	1.82±0.56a	1.36±0.02D	2.53±0.76a	1.68±0.39C	2.34±0.79a	1.80±0.57B	2.88±0.52a	1.83±0.51B	1.98±0.13a	1.94±0.04A
C16:0	21.22±0.07c	12.65±0.01D	22.17±0.08bc	12.90±0.26D	22.67±0.31b	14.06±0.13C	22.97±0.06b	17.23±0.06B	23.87±1.09a	21.37±0.11A
C16:1n-7	0.34±0.03d	0.34±0.02E	0.30±0.02d	0.48±0.02D	1.02±0.32b	0.65±0.02C	2.02±0.24a	1.05±0.02B	2.04±0.06a	1.56±0.12A
C17:0	0.43±0.02bc	0.44±0.02A	0.44±0.01b	0.39±0.02B	0.41±0.03c	0.33±0.01C	0.52±0.01a	0.38±0.03B	0.37±0.02d	0.47±0.01A
C17:1	0.82±0.23ab	0.61±0.11BC	0.93±0.28ab	1.27±0.14A	1.16±0.25a	0.74±0.19B	0.92±0.18ab	0.46±0.11C	0.65±0.03b	0.47±0.01C
C18:0	10.00±0.08e	13.24±0.03E	10.58±0.02d	15.50±0.06D	10.95±0.28c	17.04±0.11C	11.42±0.34b	17.39±0.05B	13.48±1.35a	17.67±0.40A
C18:1n-9	13.93±0.03b	13.22±0.02D	14.21±0.78b	13.65±0.50CD	14.66±0.24b	14.10±0.07C	16.42±0.28a	14.65±0.06B	16.89±0.85a	15.40±0.38A
C18:1n-7	1.20±0.04a	1.42±0.03A	1.12±0.27a	0.90±0.19C	0.65±0.03b	0.71±0.07D	0.50±0.04b	1.13±0.04B	0.99±0.38ab	1.34±0.07A
C18:2n-6	24.23±0.07a	25.26±0.10A	23.91±0.03b	22.09±0.09B	23.48±0.58b	20.80±0.19C	22.78±0.29bc	19.73±0.13D	22.56±0.99c	17.21±0.21E
C18:3n-3	0.35±0.01c	0.35±0.02E	0.31±0.02d	0.60±0.02B	0.56±0.03b	0.51±0.02C	0.85±0.02a	0.67±0.02A	0.54±0.01b	0.45±0.02D
C20:0	0.13±0.03a	0.10±0.02AB	0.07±0.01b	0.08±0.01B	0.11±0.01ab	0.11±0.02A	0.09±0.01b	0.12±0.01A	0.07±0.01b	0.13±0.01A
C20:1n-9	0.26±0.01b	0.22±0.02B	0.17±0.02b	0.23±0.02B	0.30±0.02ab	0.27±0.02A	0.22±0.02b	0.27±0.02A	0.37±0.12a	0.20±0.03C
C20:2n-6	0.96±0.02c	0.76±0.04E	1.11±0.03d	0.98±0.02D	1.14±0.03b	1.14±0.02B	1.23±0.07a	1.07±0.02C	1.08±0.05b	1.24±0.02A
C20:3n-6	0.95±0.01d	0.83±0.02E	1.00±0.03c	1.13±0.02D	1.28±0.03b	1.43±0.01C	1.38±0.07a	1.78±0.05B	0.99±0.05cb	1.96±0.02A
C20:4n-6	12.93±0.14a	17.88±0.08A	11.71±0.03b	17.40±0.15B	10.72±0.41c	16.08±0.12C	8.92±0.40d	13.64±0.10D	8.67±0.41d	11.22±0.17E
C20:5n-3	5.65±0.06a	5.84±0.07A	5.23±0.02b	5.48±0.07B	4.44±0.15c	5.16±0.02B	3.48±0.10d	4.59±0.04C	2.88±0.14e	3.59±0.09D
C22:5n-3	2.24±0.03a	2.96±0.03A	2.12±0.02b	2.73±0.02B	1.76±0.07d	2.72±0.02B	1.87±0.08c	1.91±0.07C	1.08±0.01e	1.56±0.03D
C22:6n-3	1.60±0.14a	1.92±0.03A	1.45±0.01b	1.61±0.06B	1.37±0.08c	1.59±0.03B	1.08±0.04d	1.24±0.01C	0.76±0.01e	1.08±0.02D
SFA[c]	34.30±0.30d	28.25±0.27E	36.22±0.98c	31.25±0.95D	36.84±0.73c	33.82±0.28C	38.31±0.35b	37.54±0.43B	40.03±0.50a	42.26±0.36A
PUFA	48.91±0.45a	55.79±0.37A	46.84±0.14b	52.03±0.22B	44.75±0.21c	49.43±0.34C	41.59±0.82d	44.63±0.12D	38.56±1.65e	38.31±0.53E
MUFA	16.79±0.16a	15.96±0.09B	16.94±0.77a	16.72±0.56C	18.41±0.48b	16.75±0.07D	20.10±0.49c	17.83±0.06B	21.41±1.28d	19.43±0.21A

[a] Results were expressed as means ± SE, data were means of three replicates.
[b] Values in the same column with different letters were significantly different ($p<0.05$), male: a-e, female: A-E.
[c] SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids.
[d] "-" : undetected.

In terms of fatty acids composition of intramuscular phospholipids of Inra rabbit, SFA among the muscles were mainly composed of palmitic (C16:0) and stearic (C18:0), MUFA were mainly represented by oleic (C18:1), whereas PUFA consisted of linoleic (C18:2) and arachidonic acid (C20:4). According to CAMBERO *et al*. (1991), the C16:0, C18:0, C18:1 and C18:2 were together representing more than 70% of the total fatty acids. A higher percentage of PUFA characterized the fatty acids composition of phospholipids (KANATT *et al*., 2006). In our study, the percentage of PUFA in males and females were accounted for 37.56-52.22% and 32.94-55.79% respectively in the intramuscular phospholipids during growth period from 35 d to 90 d. Long chain n-3 and n-6 PUFA were mainly found in phospholipids (ENSER *et al*., 2000; COOPER *et al*., 2004), which was also in good agreement with our investigation. However, the fatty acids composition was rarely detected in different rabbit muscles during different feeding days, especially on a particular rabbit species. Comparing the variety of intramuscular phospholipids from LD, LL and AM during the growth stages from 35 d to 90 d, C16:0, C18:0, palmitoleic acid methyl ester (C16:1n-7), C18:1n-9, cis-11,14-eicosadienoic acid methyl ester (C20:2n-6) and cis-8,11,14-eicosatrienoic acid methyl ester (C20:3n-6) increased significantly ($p < 0.05$) in both genders, whereas C18:2n-6, C20:4n-6, C20:5n-3, C22:5n-3 and C22:6n-3 decreased significantly ($p < 0.05$). The percentage of C16:0 and C18:0 in female-LD significantly increased ($p < 0.05$), and both C18:2n-6 and C20:4n-6 in female-LL significantly decreased ($p < 0.05$). Moreover, the percentage of C20:4n-6 in female-AM decreased faster than other samples during the test days. However, other fatty acids did not showed apparent changes. According to ALASNIER and GANDEMER (1998), the fatty acid composition of individual phospholipid classes was related to metabolic type of fibre in the rabbit, and the differences in fatty acid composition of phosphatidyl ethanolamine, phosphatidyl choline and cardiolipin explained a large part of the differences in fatty acid compositions of the total phospholipids of glycolytic and oxidative muscles.

As the major ingredient of feeds for all species, the incorporation of C18:2n-6 into the muscles, in relation to the amount in the diet, was greatest among other fatty acids. C18:2n-6 was deposited in muscle phospholipids at a high level where it and its long chain products C20:4n-6 competed well for insertion into phospholipids molecules (WOOD *et al*., 2008). Comparing the changes of fatty acids in the LD, LL and AM, the deposition rate of C16:0 was faster in the LD of Inra rabbits (both males and females) than that in the LL and AM. However, the C16:0 had lowest percentage and slowest deposition rate in AM. In terms of C18:0, LL was sequentially higher than LD and AM in male rabbits, while AM was higher than LL and LD in female rabbits. Meanwhile, the percentage and deposition rate of C18:0 in AM were also higher in females than that in males. For the C18:1n-9 percentage, LD showed the highest and fastest deposition rate in both genders. The percentage of C18:2n-6 in LL was the highest, and decreased in the maximum levels with age. According to WOOD *et al* (2008), the higher percentage of C18:2n-6 in phospholipids compared with neutral lipids in all species mean that muscle from lean animals has relatively higher percentages of this major PUFA. In addition, during the growth period from 35 d to 90 d, the initial content of C20:4n-6 in LD was higher compared with other muscles, and showed the fastest reducing rate. No significantly variation was found among other fatty acids components.

During the growth period of Inra rabbit, the n-6/n-3 values for LD, LL, and AM ranged from 4.02 to 6.61, 4.71 to 5.72 and 3.97 to 6.33 in males, and 3.88 to 5.05, 4.05 to 4.67 and 3.95 to 4.73 in females, respectively. There is an increasing recognition of the health benefits of PUFA in general, and of n-3 PUFAs in particular, because these fatty acids are essential for humans (ALESSANDRI *et al*., 1998; CONQUER *et al*., 2011). 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. Unfortunately, Western diet is very high in n-

6 fatty acids relative to n-3 fatty acids (ENSER *et al*., 2000; HARGIS and VAN ELSWYK, 1993). Nutritionist recommendations are for a ratio of n-6/n-3 PUFA of less than 5 (WOOD *et al*., 2003; KOUBA *et al*., 2003), and a ratio of n-6/n-3 below about 4.0 is required in the diet to combat various ''lifestyle diseases'' such as coronary heart disease and cancers (SIMOPOULOS, 2004; WILLIAMS, 2000). According to FAO/WHO, the recommended dose of essential PUFA in a healthy daily diet is 5/1 to 10/1 (n-6/n-3) (DALLE ZOTTE and SZENDRÖ, 2011), and a lower ratio is more desirable in reducing the risk of many of chronic diseases, even if the optimal ratio may vary depending on the disease under consideration (SIMOPOULOS, 2002). Therefore, it can be suggested that the intramuscular phospholipids of Inra rabbits is recommended. ALASNIER and GANDEMER (1998) reported that the phosphatidyl ethanolamine of oxidative muscles contains less 18:2n-6 and more 18:0 and long chain PUFA of the n-6 and n-3 series than that of glycolytic ones; phosphatidyl choline of oxidative muscles contains more 18:0 and less 16:0 and 18:2n-6 than that of glycolytic ones; cardiolipin of the oxidative muscles contains less 18:2 n-6 than those of the glycolytic ones. In addition, they suggested that a part of the composition difference could be related to high mitochondria content of the oxidative muscles compared to the glycolytic ones. To check this hypothesis, further investigations are required to determinate the fatty acid composition of individual phospholipid classes of both mitochondria and microsomes in rabbit muscles.

3.3. Analysis of PLSR on composition of intramuscular phospholipids

The analysis of PLSR showed that the first and second main ingredients explained 43% and 33% Y variables, respectively. From the nutritional point of view, the PUFA/SFA value is often used to evaluate the nutritional value of the meat, and higher value represents better nutritional value. However, the higher SFA + MUFA value of the meat are, the tenderness juiciness and the better flavor are (CAMERON and ENSER, 1991). On the contrary, if the content of PUFA is too high, the tenderness, flavor and juiciness of meat are poor. Hence, the SFA + MUFA value can be used to measure the quality indicators of samples after processing.

The SFA + MUFA value of intramuscular phospholipid fatty acids of Inra rabbit was located in the bottom right renderings, indicating the higher the nutritional value of the sample in the bottom right, and the PUFA/SFA values were located in the top left of the renderings (Fig. 2). Thus, the better flavor of the sample after processing is closer to the top left. On the first principal component, the composition of phospholipid fatty acids showed obviously different in ages, genders and muscles. The LD was closer to the SFA + MUFA, indicating the better phospholipid processed-flavor after processing of LD. However, the AM was closer to PUFA/SFA, indicating the better phospholipid nutritional value of AM. On the second principal component, the composition of phospholipid fatty acid of the raw material showed obviously different in ages and genders. The nutritional value of the total lipid decreased with age.

In addition, the 35 d-feedstock located in the top left oval, closely to 12 (C18: 2n-6), 18 (C20: 4n-6), 19 (C20: 5n-3), 20 (C22: 5n-3), 21 (C22: 6n-3) and other PUFAs, while the 90 d-feedstock located in the bottom right of the ellipse, closely to 6 (C16:1n-7,), 2 (C14:0), 3(C14:1n-6), 5 (C16:0), 10 (C18:1n-9) and some other saturated and monounsaturated fatty acids. Moreover, the intramuscular phospholipids of male rabbits was closely related to the 5 (C16:0) and 12 (C18:2n-6), that is the C16:0 and C18:2n-6 percentage in muscle was higher in male rabbits than that in the females. The intramuscular phospholipid of female rabbits was closely related to the percentage of 9 (C18:0), 14 (C20:0) and 18 (C20:4n-6), that is the C18:0, C20:0 and C20:4n-6 levels in muscle were higher in female rabbits than that in the males. Among the three sections, the AM had better phospholipid nutritional

value, which may be due to the higher percentage of PUFA, and the LD and LL showed better phospholipid flavor after processing, which may be due to the higher percentage of 13 (C18:3n-3) and 7 (C17:0), 15 (C20:1n-9), respectively.

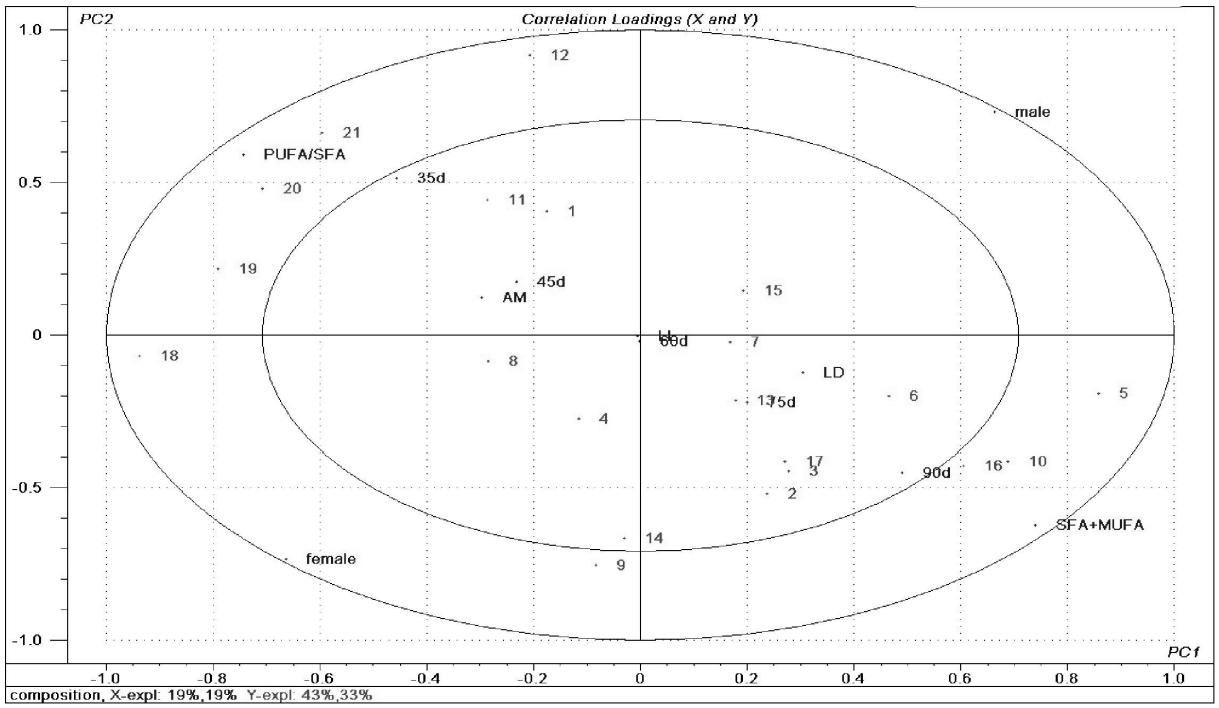

Figure 2: A PLSR correlation loadings plot for first 2 principal components (PCs). Ten 0/1 indicators variables (35 d, 45 d, 60 d, 75 d, 90 d, female, male, LD, LL, AM), SFA+MUFA, and PUFA/SFA in the X-matrix and 21 kinds of fatty acids (C12:0 - C22:6n-3 were represented by the number 1-21) in the Y-matrix. Ellipses represent R^2=0.5 (50%) and 1.0 (100%).

Overall, the composition of phospholipid fatty acids at different ages, genders and muscles showed significant difference. The effects of age, gender and muscle on the composition of phospholipid fatty acids mainly reflected on the first principal component. However, on the second principal component, only ages and genders showed the obvious difference of phospholipid fatty acids composition.

4. CONCLUSIONS

Inra rabbits are a meat source of nutritious quality, containing low content of intramuscular lipids, low ratio of n-6/n-3, whilst high content intramuscular phospholipids (% lipid). A higher content of PUFA characterised the fatty acids composition of the phospholipids, and the significantly decrease of PUFA in intramuscular phospholipids during growth were observed. Among the fatty acids from intramuscular phospholipids, SFA mainly consists of C16:0 and C18:0, MUFA consist of C18:1, and PUFA consist of C18:2 and C20:4. There is a wide variation of total lipids content and fatty acid composition at different ages, genders and muscles in Inra rabbit. By the analysis of PLSR, the nutritional value of the phospholipid fatty acids decreased with age, and the AM showed better nutritional value than LD and LL. The absolute data analysis is important for recommendations and suggestion of the consumption of dietary

phospholipids from animal sources. Further investigation is necessary to explore the properties of processing and nutritional characteristics of different sections from Inra rabbit meat.

ACKNOWLEDGEMENTS

The authors are grateful to College of Animal Science and Technology, Southwest University for the help in the meat sample supply. This study was funded by The Special Public Welfare Industry (Agriculture) Research Program of China (Grant No. 201303144), the National Rabbit Industry Technology System Program (Grant No. CARS-44D-1), the Doctoral Scientific Research Foundation of Minnan normal university (Grant No. 2006L21513), and the Program of education and scientific research for young and middle-aged teachers in Fujian Province (Grant No. JAT160296).

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Paper Received December 11, 2014 Accepted September 10, 2015