Effects of perilla (Perilla frutescens L.) seeds supplementation on performance, carcass characteristics, meat quality and fatty acid composition of rabbits.

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ABSTRACT

An experiment has been conducted to study the effects of increasing levels of Perilla frutescens L. seed (PFS) in the diet on the performance, meat quality traits, lipid oxidation and fatty acid profile of rabbit fat and meat. Thirty weaned, crossbred (Carmagnola Grey×New Zealand) rabbits aged thirty days and weighing on average, 1120±193 g, were divided into three groups of 10 (five male and five female rabbits each). Three levels of PFS (0, 5, or 10%) were included in isonitrogenous and isocaloric diets. The experimental period lasted 50 days. The performance and meat quality traits were not affected by the dietary treatments. The impact of the PFS enrichment of the diets on the oxidative stability of frozen rabbit meat was significant, but with low values and without any important effect on the meat quality. The polyunsaturated fatty acid (PUFA) concentration in the longissimus dorsi muscle and perirenal fat was significantly increased with increasing PFS inclusion, while the saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA) decreased. The n-6/n-3 PUFA ratio of the rabbit meat decreased from 6.53 in the control group, to 1.00 in the 10% PFS group. These results shown that the use of a diet supplemented with PFS is effective in reducing the saturation, atherogenic and thrombogenic indexes in the rabbit tissues.

Introduction

Rabbit meat can be enriched with n-3 polyunsaturated fatty acid (n-3 PUFA) by adding oilseed rich in α-linolenic acid (ALNA) in the form of false flax (Peiretti et al., 2007), chia (Peiretti and Meineri, 2008) and linseed (Bianchi et al., 2009). Perilla (Perilla frutescens) seeds (PFS) are a good source of the ALNA and these and other aspects of their dietary value have been studied (Longvah and Deosthale, 1991). Perilla oil, which constitutes approximately 40% of the seed weight, is primarily composed of fatty acids, such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9), linoleic acid (C18:2n-6), and ALNA (Kwak, 1994; Lee et al., 2002). The dietary intake of perilla oil containing a large amount of ALNA provides various health benefits, such as a lowering of the plasma lipid level, and has shown an increase in eicosapentaenoic and docosahexaenoic acids in the hepatic membranes of rats (Kim and Choi, 2001; Kim et al., 2004). The consumption of perilla oil has also been reported to improve learning ability and retinal functioning and suppress carcinogenesis, metastasis, thrombosis and allergies (Kinsella, 1991). The potential beneficial effects of decreasing the circulating levels of serum cholesterol and triglycerides, without toxicity, have also been shown in a short-term Wistar rats experiment (Longvah et al., 2000). The present research was designed to study the use of PFS as a dietary source of n-3 PUFA

for the production of healthy rabbit meat and to show its effect on the FA profile of the meat and perirenal fat.

Materials and methods

Animals and diets

The study was carried out at the Deptartment of Animal Sciences experimental rabbitry in Carmagnola (Turin). Thirty weaned crossbred (Carmagnola Grey×New Zealand) rabbits that are thirty days old, with a mean weight of 1120±193 g, were randomly assigned to three groups of 10 (five male and five female rabbits each) with equal initial weight variability. The animals were housed individually at a temperature of 22± 2 °C in wire cages at a height of 90 cm from the concrete floor. These groups were fed an isocaloric and isonitrogenous diet ad libitum, enriched with different levels of PFS (0%, 5% and 10%). The ingredients and composition of the experimental diets are reported in Tables 1 and 2, respectively. PFS and diet samples were analysed in duplicate for crude protein (AOAC 955.04) and ether extract (AOAC 963.15) according to the methods of the Association of Official Analytical Chemists (1990) for acid detergent fibre (ADF) and neutral detergent fibre (NDF) without sodium sulfite and α-amylase, as described by Van Soest et al. (1991) expressed exclusive of residual ash. The gross energy (GE) was determined using an adiabatic bomb calorimeter (IKA C7000, Staufen, Germany). All the diets were pelleted fresh and stored in darkness in a temperature controlled room to avoid auto-oxidation of the lipid sources.

Growth performance

The live weight and feed intake of the rabbits were recorded fortnightly during the experimental period, except for the last period, which lasted 8 days. Data on the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated.

Slaughter procedures and sample collection

No rabbits died during the trial. At the end of the experimental period, which lasted 50 days, all the rabbits were slaughtered at a mean weight of 2674±299 g in an experimental slaughterhouse without fasting. The carcasses were prepared by removing the skin, feet, paws, genital organs, urinary bladder and digestive tract, as recommended by Blasco et al. (1993). The carcass was weighed and the weights of the skin and limbs, liver, kidneys, heart, lungs and full gastrointestinal tract were recorded and expressed as a percentage of slaughter weight (SW).

Table 1
Ingredients (%) of the experimental diets.

	Perilla seed (% of diet)		
	0	5	10
Corn	14.4	18.0	17.0
Barley	20.0	18.0	16.7
Dehydrated alfalfa meal	46.0	42.0	42.0
Soybean seed meal	12.0	11.0	9.0
Palm oil	3.6	2.0	1.3
Perilla seed	0.0	5.0	10.0
Vitamin–mineral premix [a]	2.0	2.0	2.0
Lignosulphite	2.0	2.0	2.0

[a] per kg of diet: Vit. A 200 IU; α-tocopheryl acetate 16 mg; Niacine 72 mg; Vit. B_6 16 mg; Choline 0.48 mg; DL-methionine 600 mg; Ca 500 mg; P 920 mg; K 500 mg; Na 1 g; Mg 60 mg; Mn 1.7 mg; and Cu 0.6 mg.

Table 2
Chemical composition (on dry matter basis) of the perilla seed and experimental diets.

	Perilla seed	Perilla seed (% of diet)		
		0	5	10
Dry matter, %	95.3	91.5	92.1	91.8
Organic matter, %	96.2	91.0	92.1	92.5
Crude protein, %	23.9	19.5	19.5	19.4
Ether extract, %	43.0	5.1	6.6	8.1
Neutral detergent fibre, %	29.7	27.6	28.8	28.3
Acid detergent fibre, %	22.6	16.8	17.8	18.1
Gross energy, MJ/kg DM	28.0	18.4	18.8	19.4
Digestible energy [a], MJ/kg DM	–	12.2	12.0	11.9

[a] The digestible energy content of the diets was calculated according to the regression proposed by Fernández-Carmona et al. (1996).

Meat quality analysis

Sample preparation After 24 h of chilling in a refrigerated room (+4 °C), the carcasses were halved and the two longissimus dorsi (LD) muscles were excised. The left LD muscle was divided into two parts. The fore part was used to measure pH, colour and cooking losses. The hind part of the left LD and the whole right LD were vacuum-packed, frozen and stored at −20 °C for a week until their utilization. The Warner Bratzler shear force was determined using the hind part of the left LD muscle; the chemical composition and the thiobarbituric-acid reactive substances (TBARS) values were determined using the right LD muscle. The perirenal fat was vacuum-packed, frozen and stored at −20 °C for a week until analysis.

pH measurement

pH (pH24) was measured both on the LD and biceps femoris (BF) muscles by means of a Crison MicropH 2001 (Crison Instruments, Barcelona, Spain) provided with a combined electrode and an automatic temperature compensator.

Colour measurements

Meat colour was assessed on the freshly cut surface of the loin at the 7th lumbar vertebra level and on the surface of the BF muscle at room temperature (20 °C) using a Minolta CR- 331C Minolta Colorimeter (Ø 25 mm measuring area, 45° circumferential illumination/0° viewing angle geometry) with the D65 illuminant and 2° standard observer. The results were expressed in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB colour space model (CIE, 1976). Chroma [C*=(a□2+b□2)1/2] and Hue [H0=tan−1(b*/ a*)] were calculated according to Boccard et al. (1981). The colour values were obtained considering the average of three readings per meat sample.

Cooking losses

Each sample of the loin was weighed, placed in a vacuum sealed polyethylene bag, totally immersed in a constant temperature water-bath and cooked at 80 °C for 1 h (Ramírez et al., 2004) to calculate the cooking losses. After cooking, the samples were cooled under running water for 30 min. The samples were then removed from the bags, blotted and weighed. The cooking losses were expressed as a percentage of the initial weight.

Shear force

The loins, cooked as described above, were cut into rectangular cross-section strips (1 cm thick×1 cmwide×3 cm along the fibre) and were sheared perpendicular to the muscle fibre direction using an Instron 5543 equipped with aWarner– Bratzler shear device and crosshead speed set at 100mm/min (AMSA, 1995). The maximum force measured to shear the strips was expressed as Newtons (N). 2.4.6.

Chemical analyses

The chemical composition (water, ash, and crude protein (AOAC 955.04)) and ether extract (AOAC 963.15) analysis was conducted in duplicate on lyophilized samples of the right LD muscle and expressed on a fresh basis. The GE was determined by means of an adiabatic bomb calorimeter (IKA C7000, Staufen, Germany).

Lipid oxidation

Lipid oxidation was determined on the LD muscle after 3 months of storage at −20 °C using modified thiobarbituric acid (TBA) analysis according to the iron-induced thiobarbituric- acid reactive substances (TBARS) procedure described by Sárraga et al. (2006). The assay was performed at 30 min of incubation and absorbance was read at 532 nm. Liquid malonaldehyde bis (diethyl acetal, MDA) (Aldrich Chemical Co. Ltd., Dorset, UK) was used as the standard to determine the linear standard response and recovery. The TBARS values were expressed as mg of MDA per kilogram of muscle tissue.

Fatty acid determination

Lipid extractionwas performed on the PFS, the diets and the perirenal fat and LDmuscle sampleswere carried out according to Hara and Radin (1978), and the transesterification of the FA was carried out according to Christie (1982) with the modifications described by Chouinard et al. (1999). The FA content in the perilla seed oilwas the average of two replicates while in the experimental diets it was the average of three replicates. The FA were analysed as the methyl esters. The analysis was carried out by gas chromatography, using a Dani GC 1000 DPC (Dani Instruments S.P.A., CognoMonzese, Italy), equippedwith a fused silica capillary column—Supelcowax-10 (60 m×0.32mm (i.d.), 0.25 μm). The PTV injection and flame ionization detector (FID) ports were set at 245 °C and 270 °C, respectively. The oven temperature programme was set at 50 °C for the first minute, increased at a rate of 5 °C/min to 230 °C, where it remained for 24 min. The carrier gas was hydrogen. One microlitre was injected using a Dani ALS 1000 auto sampler with a 1:50 split ratio. The peak area was measured using a Dani Data Station DDS 1000, and each peak was identified and quantified according to pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

Calculation and statistical analysis

The saturation (S/P), atherogenic (AI) and thrombogenic (TI) indexes were calculated according to Ulbricht and Southgate (1991) as follows:

$$S/P = (C14:0 + C16:0 + C18:0) / \Sigma MUFA + \Sigma PUFA$$

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / [\Sigma MUFA + \Sigma(n-6) + \Sigma(n-3)]$$

$$TI = (C14:0 + C16:0 + C18:0) / [0.5 \times \Sigma MUFA + 0.5 \times \Sigma(n-6) + 3 \times \Sigma(n-3) + \Sigma(n-3) / \Sigma(n-6)]$$

where MUFA and PUFA are monounsaturated fatty acids and polyunsaturated fatty acids, respectively. The statistical analyses were performed using the SPSS (1999) software package (version 11.5.1 for Windows, SPSS Inc., USA). Analysis of variance was used to evaluate the effects of different concentrations of PFS on the performance, carcass characteristics, meat composition and fatty acid profile of the meat and fat of the rabbits. The differences were tested using Duncan's New Multiple Range Test.

Results and discussion

Composition and fatty acid profile of the PFS and the diets

Perilla seeds are rich in fat and protein (Table 2) and PFS is higher in dry matter, organic matter, crude protein and GE contents than the plant during the growth cycle, while the ether extract content was from twentyfold to tenfold more in the seed than in the plant during the growth cycle (Peiretti, 2011).

Table 3
Fatty acid contents (g/100 g of total FA) of perilla seed oil.

	This study[a]	Huang et al. (1997)	Ihara et al. (1998)	Longvah et al. (2000)	Ide et al. (2000)
C14:0	0.1 ± 0.00	0.1	–	–	0.1
C16:0	6.4 ± 0.03	7.9	6.2	8.9	6.3
C16:1	0.2 ± 0.00	0.2	–	–	0.2
C18:0	1.7 ± 0.00	1.8	1.4	3.9	2.0
C18:1n-9	12.1 ± 0.09	20.7	18.5	12.5	20.9
C18:2n-6	16.2 ± 0.01	27.1	15.5	17.8	13.3
C18:3n-3	62.0 ± 0.09	51.0	58.4	56.9	56.7
C20:1	0.1 ± 0.00	0.3	–	–	–
C20:4n-6	–	0.1	–	–	–
C20:5n-3	–	0.3	–	–	–
C22:6n-3	–	0.5	–	–	–

[a] Means of two replicates (means ± S.E.).

The PFS showed a high percentage of ALNA and a low percentage of C18:1n-9, while C18:2 n-6, C16:0, and other minor FA (Table 3) were in the range reported in literature for rat feeding trials (Huang et al., 1997; Ihara et al., 1998; Longvah et al., 2000; Ide et al., 2000). Because of the high fat content in the seeds, the diets were balanced using different proportions of palm oil and PFS (Table 1). The ether extract content increased with increasing PFS inclusion level. The ALNA contents in the experimental diets increased while C18:2n-6 and other FA decreased as PFS inclusion increased (Table 4). The trend of ALNA was similar to those found in mixed feed with Chia (Salvia hispanica L.) seed (Peiretti and Meineri, 2008) and false flax (Camelina sativa L.) seed supplements (Peiretti et al., 2007).

Growth performance and carcass traits

The productive performance and carcass characteristics are given in Table 5. There were no differences among the groups in consumption of the diets or productive performance. Longvah et al. (2000) did not find differences in food intake, body weight gain, or feed efficiency of rats fed perilla seed oil or groundnut oil. No effects on growth and carcass traits were found with a 3% linseed oil diet (Bielanski and Kowalska, 2008), a 3% extruded linseed diet (Kouba et al., 2008), or 3 to 9% whole linseed diets (Bianchi et al., 2009). Contrary to this, others found an increase in growth rate in 35–84 day old rabbits that were fed different dietary ratios of sunflower and linseed oils (Eiben et al., 2010). Animals fed a diet enriched with animal fat showed higher lumbar circumference, liver weight and carcass and retail cut weights than animals fed linseed or sunflower seed oil supplemented diets (Pla et al., 2008). The only carcass parameter that differed in the current study was the cumulative weight of the heart and lungs. This was probably due to the increased fat deposition in the pericardic zone as a consequence of the increased ether extract content of the mixed feed with the increasing perilla seed supplementation and to the higher digestibility of the ether extract as reported by Peiretti et al. (2010).

Table 4
Fatty acid contents (g/100 g of total FA; means ± S.E.) of the experimental diets.

	Perilla seed (% of diet)		
	0	5	10
C14:0	0.71 ± 0.003 [a]	0.45 ± 0.03	0.18 ± 0.003
C16:0	27.12 ± 0.14	23.28 ± 1.13	13.34 ± 0.15
C16:1n-9	0.63 ± 0.005	0.33 ± 0.01	0.32 ± 0.01
C16:1n-7	0.19 ± 0.009	0.17 ± 0.005	0.30 ± 0.023
C18:0	6.89 ± 0.09	4.57 ± 0.20	2.84 ± 0.01
C18:1n-9	31.04 ± 0.26	26.83 ± 0.93	18.00 ± 0.10
C18:1n-7	1.12 ± 0.01	0.90 ± 0.02	0.89 ± 0.07
C18:2n-6	25.72 ± 0.33	24.17 ± 0.35	22.18 ± 0.06
C18:3n-3	5.19 ± 0.06	18.06 ± 2.65	40.97 ± 0.20
C18:4n-3	0.61 ± 0.01	0.61 ± 0.04	0.56 ± 0.03
C20:0	0.38 ± 0.003	0.35 ± 0.013	0.24 ± 0.003
C20:1n-9	0.41 ± 0.012	0.29 ± 0.005	0.20 ± 0.011

[a] Means of three replicates.

Table 5
Productive performance and carcass characteristics (means ± S.E.) of rabbits fed the experimental diets.

	Perilla seed (% of diet)		
	0	5	10
Numbers of animals	10	10	10
IBW, g	1098 ± 64	1109 ± 53	1156 ± 58
ADFI, g	120.4 ± 6.5	111.6 ± 3.6	119.3 ± 6.1
ADG, g	31.3 ± 1.5	30.9 ± 1.6	31.1 ± 1.7
FCR,	3.9 ± 0.1	3.7 ± 0.2	3.9 ± 0.2
Slaughter weight (SW), g	2662 ± 112	2653 ± 62	2712 ± 98
Carcass weight, g	1608 ± 61	1651 ± 37	1637 ± 69
Carcass yield, %	59.0 ± 0.7	60.3 ± 0.6	60.0 ± 0.5
Liver, g/100 g SW	2.6 ± 0.1	2.8 ± 0.2	2.5 ± 0.1
Kidneys, g/100 g SW	0.56 ± 0.02	0.56 ± 0.01	0.56 ± 0.03
Heart and lungs, g/100 g SW	1.04 ± 0.06[a]	1.13 ± 0.04[a]	1.25 ± 0.02[b]
Skin and limbs g/100 g SW	18.2 ± 0.1	18.2 ± 0.4	18.0 ± 0.4
Full gastointestinal tract g/100 g SW	16.8 ± 0.7	16.0 ± 0.5	16.1 ± 0.4

IBW: initial body weight.
ADFI: average daily feed intake.
ADG: average daily gain.
FCR: feed conversion ratio.
[ab] Means in the same row with unlike superscripts differ ($P<0.05$).

Meat quality traits

The pH24, colour parameters, cooking losses, and shear force of the LD muscle and the pH24 and colour parameters of the BF muscle are reported in Table 6. The LD and BF muscles were not affected by the

dietary treatment for any of these parameters. The ultimate pH of the meat was identical for the different groups. These results are consistent with Castellini et al. (1998), who did not find any variations in colour parameters ascribable to the diet in the meat of rabbits fed diets enriched ith n-3 PUFA and supplemented with vitamin E. Contrary to this, Oliver et al. (1997) found that meat redness tends to increase in rabbits fed with a vegetable fat enriched diet versus rabbits fed a control diet without soybean oil.

Table 6
Meat traits (means ± S.E.) of rabbits fed experimental diets.

	Perilla seed (% of diet)		
	0	5	10
Numbers of animals	10	10	10
Longissimus dorsi muscle			
pH_{24}	5.55 ± 0.01	5.57 ± 0.01	5.55 ± 0.01
L*	62.0 ± 1.0	61.9 ± 0.8	61.6 ± 01.0
a*	12.9 ± 0.6	12.3 ± 1.0	12.5 ± 0.8
b*	5.2 ± 0.5	5.9 ± 0.4	5.8 ± 0.3
Chroma	14.0 ± 0.6	13.7 ± 0.9	13.8 ± 0.7
Hue	22.1 ± 1.9	26.7 ± 2.2	25.4 ± 1.7
Cooking losses (%)	27.5 ± 0.4	27.1 ± 0.8	27.7 ± 0.6
Shear force (N)	17.6 ± 2.1	17.8 ± 1.8	21.1 ± 2.2
Biceps femoris muscle			
pH_{24}	5.68 ± 0.02	5.69 ± 0.01	5.71 ± 0.01
L*	58.7 ± 1.1	60.3 ± 1.2	59.3 ± 1.3
a*	15.4 ± 0.9	14.5 ± 1.4	16.2 ± 1.6
b*	5.9 ± 0.8	5.1 ± 0.4	5.3 ± 0.3
Chroma	16.6 ± 0.9	15.5 ± 1.4	17.2 ± 1.4
Hue	20.7 ± 2.4	20.2 ± 1.6	19.9 ± 2.6

L*: lightness.
a*: redness.
b*: yellowness.

In the study of Cavani et al. (2003) the meat of rabbits on an 8% whole linseed diet exhibited higher redness (a*) and lower lightness (L*) when compared to those of the untreated controls. Pla et al. (2008) found a minor effect of differing dietary n-3 and n-6 FA contents on redness but agreed with the data of Eiben et al. (2010), who found that the carcass was less light (L*) from the oil-rich diets and less yellow (b*) from the linseed oil than sunflower oil addition. Bianchi et al. (2009) found a significant influence of the diets containing 6 and 9% of whole linseed supplementation on only meat redness (a*). These values were lower than those obtained in the meat of rabbit fed control and 3% whole linseed diets. The pH24, cooking losses, and other colour parameters did not change significantly. Oliver et al. (1997) found that an animal fat enriched diet had a significant effect on the pH24 and colour of the LD muscle and on the perirenal fat colour when compared to vegetable oil (soybean oil) enriched and control diets. In particular, the pH24 of the LD muscle was higher in rabbits fed an animal fat enriched diet than in the other two groups. The redness (a*) and yellowness (b*) values of the LD muscle were higher in the rabbits fed vegetable fat than the other two groups. Perirenal fat of rabbits fed vegetable fat was darker (L*) than the other two groups while the yellowness (b*) of the fat was lowest in the group fed an animal fat enriched diet. The proximate composition and gross energy of the LD muscle were not significantly affected by the dietary treatment (Table 7). This is in agreement with the results of Dal Bosco et al.

(2004) who used dietary ALNA and vitamin E and Peiretti et al. (2007) who used different levels of false flax seed in diets for fattening rabbits.

Oxidative stability of the meat

The average TBARS values of MDA after three months storage at −20 °C increased with increasing perilla seed supplementation (Pb0.05) (Table 7). However, the oxidative stability of the LD muscle after three months of storage at −20 °C was lower than that found after 1 and 8 days of storage at 4 °C by Dal Bosco et al. (2004). Kouba et al. (2008) did not find any adverse effect of a high n-3 PUFA level of muscle and its susceptibility to oxidation, even with a low quantity of vitamin E in a linseed diet.

Table 7
Chemical composition (on a dry matter basis; means ± S.E.) and gross energy of the *longissimus dorsi* muscle of rabbits after 1 day of storage at 4 °C and oxidative stability (mg MDA/kg muscle tissue) of the *longissimus dorsi* muscle after 3 months of storage at −20 °C.

	Perilla seed (% of diet)		
	0	5	10
Numbers of animals	10	10	10
Dry matter, %	24.97 ± 0.16	25.32 ± 0.24	25.13 ± 0.18
Protein, %	22.99 ± 0.13	22.99 ± 0.17	22.80 ± 0.15
Ether extract, %	0.66 ± 0.04	0.85 ± 0.06	0.84 ± 0.08
GE, MJ/kg	5.53 ± 0.07	5.52 ± 0.04	5.57 ± 0.07
TBARS, mg MDA/kg	0.21 ± 0.01[a]	0.47 ± 0.06[b]	0.57 ± 0.07[b]

[a,b]Means in the same row with unlike superscripts differ ($P<0.05$).

Bianchi et al. (2009) found no differences in the susceptibility to lipid oxidation in the LD muscle of growing rabbits fed a commercial diet or experimental diets containing 3, 6 or 9% whole linseed without vitamin E supplementation. Castellini et al. (1998) found that feeding an n-3 PUFA enriched diet, supplemented with a high amount of vitamin E, significantly lowered the TBARS level in the LD muscle. Although some components of P. frutescens can prevent superoxide formation in human promyelocytic leukemia HL-60 cells (Nakamura et al., 1998) and lipid peroxidation in mice (Terao et al., 1991), it seems that PFS does not favour the oxidative stability of meat when rabbits are fed diets with higher amounts of n-3 PUFA.

Fatty acid profile of the meat

The FA composition of the LD muscle is shown in Table 8. As expected, the FA composition of the diet influenced the FA composition of the meat. The ALNA content increased (Pb0.05) from 3.0% in the meat of rabbits fed the control diet, to 14.7% and 19.7% in the meat of the rabbits fed the 5% and 10%

PFS diets, respectively. Conversely, a decrease with an increasing PFS inclusion level was found for C16:0, C17:0, C18:0, C18:1n-9 and C18:1n-7. No significant differences were detected among the treatments for the other FA. Similar FA trends have been found in the meat of rabbits fed increasing levels of false flax seed (Peiretti et al., 2007) and chia seed (Peiretti and Meineri, 2008).

Table 8
Fatty acid composition (g/100 g of total FA; means ± S.E.) and indexes related to human health in the *longissimus dorsi* muscle of rabbits fed the experimental diets.

	Perilla seed (% of diet)		
	0	5	10
Numbers of animals	10	10	10
C14:0	2.19 ± 0.10	1.96 ± 0.09	1.94 ± 0.10
C15:0	0.32 ± 0.06	0.23 ± 0.07	0.36 ± 0.01
C16:0	29.88 ± 0.48^{a}	26.28 ± 0.52^{b}	23.74 ± 0.11^{c}
C16:1n-9	0.30 ± 0.06	0.28 ± 0.06	0.21 ± 0.06
C16:1n-7	4.14 ± 0.29	3.21 ± 0.30	3.25 ± 0.33
C17:0	0.60 ± 0.04^{a}	0.43 ± 0.07^{b}	0.46 ± 0.02^{b}
C18:0	7.83 ± 0.17^{a}	6.84 ± 0.18^{b}	6.44 ± 0.25^{b}
C18:1n-9	29.68 ± 0.36^{a}	24.75 ± 0.30^{b}	22.88 ± 0.69^{c}
C18:1n-7	1.61 ± 0.12^{a}	1.03 ± 0.02^{b}	1.02 ± 0.03^{c}
C18:2n-6	17.41 ± 0.61	17.40 ± 0.37	17.72 ± 0.45
C18:3n-3	3.05 ± 0.10^{a}	14.71 ± 0.87^{b}	19.66 ± 0.79^{c}
C20:4n-6	2.39 ± 0.22	1.95 ± 0.18	1.76 ± 0.27
Other	0.61 ± 0.40	0.94 ± 0.23	0.58 ± 0.04
SFA	40.82 ± 0.53^{a}	35.74 ± 0.59^{b}	32.93 ± 0.24^{c}
MUFA	35.72 ± 0.53^{a}	29.27 ± 0.55^{b}	27.35 ± 1.02^{b}
PUFA	22.85 ± 0.81^{a}	34.06 ± 1.04^{b}	39.14 ± 0.99^{c}
PUFA n-3	3.05 ± 0.10^{a}	14.71 ± 0.87^{b}	19.66 ± 0.79^{c}
PUFA n-6	19.80 ± 0.78	19.35 ± 0.37	19.48 ± 0.67
n-6/n-3	6.53 ± 0.29^{a}	1.35 ± 0.09^{b}	1.00 ± 0.06^{b}
S/P	0.68 ± 0.02^{a}	0.56 ± 0.02^{b}	0.48 ± 0.01^{c}
Atherogenic index	0.66 ± 0.02^{a}	0.54 ± 0.02^{b}	0.47 ± 0.01^{c}
Thrombogenic index	1.08 ± 0.02^{a}	0.51 ± 0.03^{b}	0.39 ± 0.01^{c}

^{a,b,c} Means in the same row with unlike superscripts differ ($P<0.05$).
SFA: Saturated Fatty Acid.
MUFA: Monounsaturated Fatty Acid.
PUFA: Polyunsaturated Fatty Acid.
PUFA n-3: Polyunsaturated Fatty Acid series n-3.
PUFA n-6: Polyunsaturated Fatty Acid series n-6.
n-6/n-3: PUFA n-6/PUFA n-3 ratio.
S/P index (saturated fatty acid/unsaturated fatty acid ratio).

Fatty acid profile of the perirenal fat

The perirenal fat FA profile reflected the dietary FA composition and the increased ALNA content in the diets with 5 and 10% of PFS (Table 4). This resulted in higher percentages of these FA in the perirenal fat of the rabbits fed these diets than those fed the control diet (Table 9). TheALNAcontentwas 4.24% in the rabbits fed the control diet and 20.74 and 25.45% in those fed the 5 and 10% PFS diets, respectively. The palmitic acid, stearic acid, and oleic acid contents decreased (Pb0.05) with increasing levels of PFS and this reflected the same trend as these FA in the diets. Other FA, such as C14:0, C16:1n-9, C16:1n-7, C18:1n-7, and C20:1n-9 were significantly lower in the perirenal fat of the rabbits fed supplemented

diets than those of the rabbits fed the control diet. No significant differences were detected among the treatments for the other FA.

Relationship to nutritional quality

The saturated fatty acid (SFA), MUFA, PUFA, n-6 PUFA and n-3 PUFA contents and their n-6/n-3 ratio and saturation (S/ P), atherogenic (AI) and thrombogenic (TI) indexes for the LD muscle and perirenal fat are reported in Tables 8 and 9. These indexes can be used to evaluate the nutritional quality of the meat and perirenal fat of rabbits and to describe the dietetic value for human consumption.

Table 9
Fatty acid composition (g/100 g of total FA; means ± S.E.) and indexes related to human health in the perirenal fat of rabbits fed the experimental diets.

	Perilla seed (% of diet)		
	0	5	10
Numbers of animals	10	10	10
C14:0	2.21 ± 0.07^{a}	1.62 ± 0.09^{b}	1.55 ± 0.04^{b}
C15:0	0.42 ± 0.01	0.38 ± 0.01	0.39 ± 0.02
C16:0	28.87 ± 0.69^{a}	22.75 ± 0.33^{b}	20.81 ± 0.21^{c}
C16:1n-9	0.38 ± 0.01^{a}	0.24 ± 0.03^{b}	0.25 ± 0.01^{b}
C16:1n-7	3.12 ± 0.25^{a}	1.84 ± 0.20^{b}	1.75 ± 0.18^{b}
C17:0	0.54 ± 0.01^{a}	0.46 ± 0.02^{b}	0.47 ± 0.01^{b}
C18:0	6.56 ± 0.12^{a}	5.27 ± 0.14^{b}	5.22 ± 0.16^{b}
C18:1n-9	32.50 ± 0.49^{a}	24.90 ± 0.33^{b}	21.86 ± 0.45^{c}
C18:1n-7	1.25 ± 0.05^{a}	0.98 ± 0.01^{b}	0.97 ± 0.02^{b}
C18:2n-6	19.47 ± 0.48	20.29 ± 0.27	20.87 ± 0.55
C18:3n-3	4.24 ± 0.09^{a}	20.74 ± 0.84^{b}	25.45 ± 0.69^{c}
C20:1n-9	0.36 ± 0.02^{a}	0.17 ± 0.03^{b}	0.17 ± 0.03^{b}
Other	0.08 ± 0.04	0.36 ± 0.19	0.24 ± 0.04
SFA	38.60 ± 0.85^{a}	30.48 ± 0.41^{b}	28.43 ± 0.36^{c}
MUFA	37.25 ± 0.59^{a}	27.96 ± 0.50^{b}	24.83 ± 0.61^{c}
PUFA	23.71 ± 0.57^{a}	41.03 ± 0.72^{b}	46.33 ± 0.89^{c}
PUFA n-3	4.24 ± 0.09^{a}	20.74 ± 0.84^{b}	25.45 ± 0.69^{c}
PUFA n-6	19.47 ± 0.48^{a}	20.29 ± 0.27^{ab}	20.87 ± 0.55^{b}
n-6/n-3	4.60 ± 0.06^{a}	0.99 ± 0.05^{b}	0.82 ± 0.03^{c}
S/P	0.62 ± 0.02^{a}	0.43 ± 0.01^{b}	0.39 ± 0.01^{b}
Atherogenic index	1.10 ± 0.04^{a}	0.75 ± 0.02^{b}	0.67 ± 0.01^{c}
Thrombogenic index	0.92 ± 0.03^{a}	0.34 ± 0.01^{b}	0.28 ± 0.01^{c}

[a,b,c] Means in the same row with unlike superscripts differ ($P<0.05$).
SFA: Saturated Fatty Acid.
MUFA: Monounsaturated Fatty Acid.
PUFA: Polyunsaturated Fatty Acid.
PUFA n-3: Polyunsaturated Fatty Acid series n-3.
PUFA n-6: Polyunsaturated Fatty Acid series n-6.
n-6/n-3: PUFA n-6/PUFA n-3 ratio.
S/P index (saturated fatty acid/unsaturated fatty acid ratio).

The meat and perirenal fat SFA and MUFA contents decreased while the PUFA content increased with increasing PFS inclusion levels. The percentage variations of SFA, MUFA and PUFA in the perirenal fat among groups were higher than those found in the LD muscle. These results are in agreement with the findings of other authors who found that the FA composition of intramuscular fat can vary less than that of the separable fat depots such as perirenal fat (Oliver et al., 1997; Bernardini et al., 1999; Peiretti et al., 2007; Peiretti and Meineri, 2008). The n-3 PUFA content of these tissues showed the greatest variations and the highest value was obtained in the tissues of the rabbits fed 10% PFS because of the high ingestion of ALNA. The effectiveness of ALNA rich oilseed in increasing the PUFA and ALNA

contents of rabbit meat has been reported in several studies (Dal Bosco et al., 2004; Bianchi et al., 2006; Peiretti et al., 2007; Peiretti and Meineri, 2008). The n-6/n-3 PUFA ratio decreased from 6.53 to 1.00 in the meat and from4.60 to 0.82 in the perirenal fat of the rabbits fed the control diet and the 10% PFS diet, respectively. Similarly, Peiretti et al. (2007) and Peiretti andMeineri (2008) found that false flax seed and chia, when fed to rabbits, significantly increased the PUFA content and lowered the n-6/n-3 ratios. The ratios found in these studieswere lower than those found in the meat of rabbits fed traditional diets, and ranged from11 to 11.6 in the hind legmeat (Dalle Zotte, 2002) to16.9intheloin(Colin et al., 2005). The S/P ratio, AI and TI of the meat and perirenal fat significantly decreased with an increasing PFS inclusion level (Tables 8 and 9). This is in agreement with other studies that found that rabbits fed oilseed showed a decreasing trend of these indexes in the muscle and perirenal fat and the values were similar to those found in the present study (Peiretti et al., 2007; Peiretti and Meineri, 2008).

Conclusions

PFS can be included in rabbit diets at levels up to 10% without any adverse effects on the performance, physical characteristics and chemical composition of the meat. PFS supplementation is effective in improving theALNA content, decreasing the n-6/n-3 ratio and reducing the P/S, AI and TI indexes of meat, thus constituting an important nutritional benefit for human beings. Moreover, the impact of PFS diet enrichment on the oxidative stability of frozen rabbit meat is low.

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