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Fatiha Benatmane, Maryline Kouba, Ahcène Youyou, Jacques Mourot. Effect of a linseed diet on lipogenesis, fatty acid composition and stearoyl-CoA-desaturase in rabbits. *animal*, Cambridge University Press (CUP), 2011, 1751-7311 (12), pp.1993-2000. <10.1017/S1751731111001145>. <hal-00730165>

**HAL Id: hal-00730165**

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Submitted on 8 Mar 2013

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# Effect of a linseed diet on lipogenesis, fatty acid composition and stearoyl-CoA-desaturase in rabbits

F. Benatmane<sup>1,2</sup>, M. Kouba<sup>2†</sup>, A. Youyou<sup>3</sup> and J. Mourot<sup>2</sup>

<sup>1</sup>Faculté des Sciences Biologiques et Agronomiques, Département d'agronomie, BP17, Tizi-Ouzou 15000, Algeria; <sup>2</sup>UMR 1079 INRA, Agrocampus Ouest, Systèmes d'Élevage Nutrition Animale et Humaine, F-35590 Saint Gilles, France; <sup>3</sup>Département de Technologie Alimentaire, Institut National d'Agronomie, El Harrach 16200, Alger, Algeria

(Received 13 October 2010; Accepted 14 June 2011)

*The aim of the study was to examine the effect of a linseed diet on meat quality and on lipogenesis in rabbits. Twelve rabbits were fed a control or a linseed diet. There was no diet effect on growth, food consumption, carcass characteristics and meat ultimate pH and colour. Feeding the linseed diet increased the n-3 polyunsaturated fatty acids (PUFA) levels in perirenal and interscapular fats, in the Longissimus dorsi muscle and in the liver. The linseed diet produced lower linoleic acid/ $\alpha$ -linolenic acid ratios in adipose tissues and in the Longissimus dorsi muscle, but not in the liver. Diet did not affect lipogenic enzyme activities in the Longissimus dorsi muscle, whereas the linseed diet decreased the lipogenic potential in perirenal and interscapular fats, and in the liver. Feeding rabbits with a high n-3 PUFA diet led to a decrease in the oxidative stability of perirenal fat and the Longissimus dorsi muscle, and to an inhibition of stearoyl-CoA-desaturase activity in liver and in adipose tissues, but not in muscle.*

**Keywords:** lipogenesis, rabbit, linseed, stearoyl-CoA-desaturase, fatty acids

## Implications

Rabbit meat consumption in France is approximately 1.5 kg/person per year, and hence it is worthwhile to improve its nutritional value. This study reports that inclusion of linseed in rabbit diet is a good way to improve this value. Our results will be useful to a future marketing of rabbit meat enriched in n-3 polyunsaturated fatty acids in France.

## Introduction

In developed countries, people tend to exercise control over their fat intake. Therefore, one of the main aims of meat research is to produce dietetic and healthy meat. Rabbit meat presents many positive dietetic characteristics such as low lipid and cholesterol levels and high polyunsaturated fatty acid (PUFA) content (Dalle Zotte, 2002; Hernández, 2008).

In rabbits, as in other monogastrics, it appears that the fatty acid (FA) composition of tissues reflects that of ingested lipids (Maertens, 1998; Mourot and Hermier, 2001). Thus, rabbit meat consumption could be healthy for consumers, as manipulation of rabbit diet seems relatively easy in order to increase the level of n-3 PUFA, conjugated linoleic acid (CLA) or vitamin E (Hernández, 2008).

Humans are not able to synthesise n-3 and n-6 PUFA but can synthesise long-chain n-6 and n-3 FAs from dietary precursors (18:2n-6 and 18:3n-3, respectively) that must be supplied by the diet. Numerous epidemiological and clinical studies have documented the health benefits of n-3 PUFA in human nutrition, particularly eicosapentaenoic (EPA) and docosahexanoic (DHA) acids. The best-known effect of these FAs is the control of cardiovascular diseases (Conquer and Holub, 1998). The key enzymes involved in the formation of these long-chain FAs are  $\Delta$ 6- and  $\Delta$ 5-desaturases, but the conversion is very limited (Alessandri *et al.*, 2009). Therefore, one way to increase EPA and DHA contents in the human diet is to produce food, meat in particular, with a high content of these FAs (Ailhaud *et al.*, 2006).

The relative importance of lipogenic enzyme activities in the liver and adipose tissues is dependent on the age of animals. During growth, the liver is the main site of lipogenesis in young rabbits, but in adults the major site is the adipose tissue (Vézinhet and Nougès, 1977; Gondret *et al.*, 1997; Gondret, 1999).

However, PUFA have a high propensity to oxidise. It is well known that tissue oxidation is one of the main mechanisms by which food quality deteriorates. Lipid oxidation, which can lead to rancidity and colour deterioration, is a major problem in rabbit meat because of its high content of PUFA (Liu *et al.*, 2009). Indeed, compared with other species, the

† E-mail: maryline.kouba@agrocampus-ouest.fr

thiobarbituric acid-reactive substance (TBARS) values observed in rabbit meat are generally higher than values in pork, chicken or in cattle meat (Dalle Zotte, 2004). However, the positive effect of the diet supplementation of vitamin E on the oxidative stability of lipids in rabbit meat has been shown by Corino *et al.* (1999).

The aim of this study was to evaluate the impact of a linseed diet on growth performances, lipogenesis potential, stearoyl-CoA-desaturase (SCD) (that generates monounsaturated FAs (MUFA)) and on meat quality in rabbits.

## Material and methods

### Animals and diets

Animals used in this experiment were reared and slaughtered in compliance with French regulations for the human care and use of animals in research (certificate of authorisation to experiment on living animals delivered by the French department of Agriculture to M. Kouba).

Twelve Californian × New Zealand 30-day-old weanling mixed-sex rabbits with a body weight of  $550 \pm 7.3$  g were given *ad libitum* access to a commercial diet formulated for rabbit growth. Six weeks before slaughter at 11 weeks of age, the animals were divided into two homogeneous groups (live weight and sex) with rabbits placed in individual cages. Both groups were fed *ad libitum* two different diets: a control diet (commercial diet) or a linseed diet containing 40 g of Croquelin® (Combourtillé, France) (a mix of 50% extruded linseeds, 30% wheat bran and 20% sunflower meal) per kg.

Both diets were isoenergetic (gross energy, 16.37 kJ/kg diet), and contained approximately 17% crude protein and 30 mg of vitamin E/kg diet (Table 1).

During the experimental period, feed intake and live weight were recorded individually each week. At 11 weeks of age, the animals were slaughtered by electric stunning and exsanguination in compliance with French national regulations for commercial slaughtering, but without prior fasting. After slaughter, hot carcasses were weighed and then cut into two halves according to the World Rabbit Scientific Association standard method (Blasco and Ouhayoun, 1993). The left half was weighed and cut into three anatomically defined primal cuts, which were also weighed and sampled: shoulder, leg and saddle. Liver and adipose tissues (interscapular and perirenal fat) were weighed, and a sample of the *Longissimus dorsi* muscle (LD) was taken. Samples of liver, adipose tissues and muscle were immediately assayed for enzyme activities. All the other samples were vacuum-packaged and deep frozen at  $-20^{\circ}\text{C}$  pending different chemical analyses.

### Meat quality variables

The ultimate pH was measured 24 h *post mortem* in the LD with a combined glass penetrating electrode (Ingold, Mettler Toledo, Greifensee, Switzerland) and a portable pH meter (WTW 340i, Weilheim, Germany) at the level of the fifth lumbar vertebra in the chilled carcass.

The colour of the LD was recorded 24 h *post mortem* using a CR-300 Minolta Chroma Meter (Minolta Camera Co.,

Osaka, Japan), which, at each point, gave the average of the three measurements of lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ).

### Chemical analysis of diet, muscle and fats

Diets were analysed for cellulose and crude protein ( $n \times 6.25$ ), according to the Association of Official Analytical Chemists (AOAC, 2006). The gross energy content of the diets was measured using an adiabatic bomb calorimeter (IKA, Staufen, Germany).

The lipids were extracted from samples of liver, *Longissimus dorsi* muscle, interscapular and perirenal fats and the diets using the method described in Folch *et al.* (1957) (extraction with a mixture of chloroform–methanol (2:1)). Fatty acid methyl esters were prepared with boron trifluoride methanol according to Morrison and Smith (1964) and analysed on a Perkin Elmer Autosystem XL gas chromatograph (Waltham, Massachusetts, USA) equipped with fused silica gel capillary column (0.25 mm i.d.  $\times 30$  m), filled with stationary phase (80% biscyanopropyl and 20% cyanopropylphenyl), using margaric acid (C17) as internal standard. The chromatography conditions used were: temperature programme from  $45^{\circ}\text{C}$  to  $240^{\circ}\text{C}$  at  $20^{\circ}\text{C}$  to  $35^{\circ}\text{C}/\text{min}$ . The injector and detector temperatures were maintained at  $220^{\circ}\text{C}$  and  $280^{\circ}$ , respectively. Results were expressed as percentages of the total FA content of the diet, liver, muscle and adipose tissues.

The non-polar and polar lipids were separated from the total lipid extracts of the LD on silica cartridges (Sep-Pack, Waters Corporation; Juaneda and Rocquelin, 1985).

Lipid oxidation in perirenal fat and LD was determined using a modification of the method described by Monahan *et al.* (1992), by assaying 2-TBARS as reported by Oriani *et al.* (2001). TBARS, expressed as nmol malonaldehyde (MDA)/g of tissue, were determined at 0, 60, 120, 200 and 300 min.

### Enzyme analysis

**Lipogenic enzyme activities.** Activities of the lipogenic enzymes were determined in the liver, the *Longissimus dorsi* muscle, interscapular and perirenal fats. Weighed amounts of tissues (2 g) were homogenised in a buffer containing 0.25 M sucrose and centrifuged at 100 000 g for 40 min at  $4^{\circ}\text{C}$ .

The cytosolic supernatant fraction was collected and was analysed for malic enzyme (ME) activity (EC 1.1.1.40) and glucose-6-phosphate dehydrogenase activity (G6PDH, EC 1.1.1.49) according to procedures outlined by Kouba *et al.* (1999). NADPH formation was measured at  $37^{\circ}\text{C}$  by absorbance at 340 nm. Enzyme activity was expressed as  $\mu\text{m}$  of NADPH produced per minute per gram of tissue.

The FA synthase (FAS, EC 2.3.1.85) activity was measured as described by Lavau *et al.* (1982). NADPH oxidation was determined at  $37^{\circ}\text{C}$  by absorbance at 340 nm. Enzyme activity was expressed as  $\mu\text{m}$  of NADPH used per minute per gram of tissue.

**SCD activity.** The activity of SCD ( $\Delta 9$ -desaturase, EC 1.14.19.1) was determined in the liver, the LD, and interscapular and perirenal fat homogenates obtained from the

samples removed after slaughter. Weighed amounts of tissue were homogenised in a buffer containing 0.25 M sucrose and 0.05 M potassium, and centrifuged at  $10\,000 \times g$  for 40 min at 4°C. Each incubation mixture for the measurement of SCD contained an aliquot of supernatant, phosphate buffer,  $MgCl_2$ , ATP, coenzyme A, NADH and [ $^{14}C$ ]stearic acid, as described by Kouba *et al.* (1999). The activity was determined by measuring the conversion of [ $^{14}C$ ]stearic acid into [ $^{14}C$ ]oleic acid. One unit of activity was defined as the amount of enzyme that produced 1 nmol of oleic acid per min. Enzyme activity was expressed as unit per gram of tissue.

**$\beta$ -Hydroxyacyl-CoA-dehydrogenase (HAD) activity.** The activity level of one mitochondria oxidative marker, reflecting FA  $\beta$ -oxidation,  $\beta$ -HAD (EC 1.1.1.35) was measured in the LD in compliance with the method described by Bass *et al.* (1969).

Approximately 400 mg of muscle was homogenised and sonicated in 50 vol. (wt./vol.) of ice-chilled 0.1 M phosphate buffer (pH 7.5) containing 2 mM EDTA.

After centrifugation at  $1700 \times g$  for 15 min at 4°C, the supernatant fraction was collected and used for  $\beta$ -HAD activity determination. Enzyme activity was measured at 30°C by determination of optical density every 25 s for 5 min using an automatic spectrophotometric analyser (Konelab, Thermo Electron Oy /Thermo Electron S. A., Finland). After addition of aceto-acetyl-CoA and NADH, the HAD activity was assessed at 340 nm. One unit of activity was defined as the amount of enzyme that degraded substrate per minute. Enzyme activity was expressed as  $\mu$ m of disappeared NADH/min per gram of fresh muscle.

#### Statistical analysis

Effects of diets on the different variables were examined with one-way ANOVA using SAS (SAS Institute, 1999). The effect of tissue origin on FA composition, TBARS and enzyme activities was also studied with one-way ANOVA using SAS (1999). The individual rabbit was the experimental unit for analysis of all data.

## Results

#### Chemical composition of the diets

The FA profiles of the two diets differed according to the ingredients (Table 1). As expected, the linseed diet had higher proportions of  $\alpha$ -linoleic acid (ALA; 18:3n-3; 22.4% *v.* 7.1%) and total PUFA (55.2% *v.* 41%) and lower proportions of saturated (SFA; 22.6% *v.* 35%) and MUFA (22.4% *v.* 28.1%) than the control diet. The linoleic acid/ $\alpha$ -linolenic acid ratio was decreased in linseed diet (1.45 instead of 4.75). LA (18:2n-6) concentration and proportion were similar in both diets.

#### Productive performances

Performances and carcass data are shown in Table 2. Diet had no effect on animal growth, feed intake, carcass weight, liver, saddle, shoulder, leg weights and meat quality.

**Table 1** Composition (as-fed basis) of experimental diets (g/kg)

	Control diet		Linseed diet	
<b>Ingredients</b>				
Wheat bran	280		280	
Alfalfa hay	220		220	
Sunflower meal	150		150	
Sugar beet pulp	100		100	
Lapilest <sup>® a</sup>	65		65	
Croquelin <sup>® b</sup>	–		40	
Wheat	50		50	
Sugar cane molasses	45		45	
Vegetal oil	10		10	
Pea	20		–	
Rapeseed meal	20		–	
Vitamin–mineral mix	40		40	
Vitamin E (mg/kg)	30		30	
<b>Proximate analysis</b>				
Gross energy (kJ/kg)	16.37		16.37	
Cellulose	140		140	
Crude proteins	170		172	
Fat	40		40	
<b>FA composition</b>				
	%	g/kg diet	%	g/kg diet
C16:0	25.07	5.58	18.57	4.29
C18:0	3.70	0.89	3.10	0.72
C18:1 n-9	26.95	5.99	21.48	4.96
C18:2 n-6	33.58	7.47	32.42	7.49
C18:3 n-3	7.07	1.58	22.40	5.18
$\Sigma$ SFA	35.05	7.79	22.60	5.22
$\Sigma$ MUFA	28.08	6.24	22.45	5.19
$\Sigma$ PUFA	40.96	9.12	55.22	12.76
LA/ALA	4.75		1.45	

FA = fatty acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; LA = linoleic acid; ALA =  $\alpha$ -linolenic acid.

<sup>a</sup>Lapilest<sup>®</sup>: a mix of beet pulp, grape pulp and seeds, apple marc and sunflower shells.

<sup>b</sup>Croquelin<sup>®</sup>: a mix of 50% extruded linseeds, 30% wheat bran and 20% sunflower meal.

#### Total lipid content

There was no effect of diet on the lipid percentage of the LD, liver, perirenal and interscapular fats (Table 3). The total lipid content deposited in the whole tissue (liver, perirenal and interscapular fats) was decreased by the linseed diet, compared with the control diet.

The lipid content of perirenal fat was much higher ( $P < 0.001$ ) than that of interscapular fat, regardless of the diet.

#### Lipogenic enzymes, SCD and $\beta$ -HAD activities

The lipogenic enzyme activities (ME, G6PDH and FAS) are shown in Table 4. The liver presented the highest lipogenic enzyme activities ( $P < 0.001$ ) in comparison with the muscle and the adipose tissues.

In liver, and perirenal and interscapular adipose tissues, the linseed diet led to a decrease of the activities of ME, G6PDH and FAS, regardless of the expression of these activities, per gram of tissue or for the whole tissue. However,

**Table 2** Effects of diets (n = 6 per diet) on growth performance, carcass traits, feed intake and meat quality

	Control	Linseed	r.s.d.	Diet effect
Final body weight (g)	2495	2350	103	ns
Average daily weight gain (g)	37	36	3	ns
Daily consumption (g)	98	106	6	ns
Food conversion ratio (kg food/kg gain)	2.63	2.99	0.31	ns
Carcass traits				
Hot carcass weight (g)	1370	1288	190	ns
Liver weight (g)	73	57	15	ns
Saddle weight (g)	312	281	66	ns
Shoulder weight (g)	193	172	32	ns
Leg weight (g)	222	207	34	ns
Meat quality				
Ultimate muscle pH <sup>a</sup>	5.70	5.81	0.10	ns
L*	58.91	57	1.75	ns
a*	1.94	3.01	1.10	ns
b*	2.15	2.01	0.34	ns

ns = no significant difference ( $P > 0.05$ ); L\* = lightness; a\* = redness; b\* = yellowness.

<sup>a</sup>pH measured 24 h post mortem in the *Longissimus dorsi* muscle.

**Table 3** Effect of diets (n = 6 per diet) on the lipid content (% or for whole tissue) of liver, LD and adipose tissues

	Control	Linseed	r.s.d.	Diet effect
Percentage of lipids				
LD	2.17	2.71	0.58	ns
Liver	3.65	3.86	0.27	ns
Perirenal fat	74.63	74.16	4.42	ns
Interscapular fat	59.84	51.82	6.45	ns
Lipid for whole tissue (g)				
Liver	2.77	2.18	0.29	*
Perirenal fat	24.59	18.98	5.02	*
Interscapular fat	15.13	11.09	2.81	*

LD = *Longissimus dorsi* muscle; ns = no significant difference ( $P > 0.05$ ).

\* $P < 0.05$ .

there was no diet effect on ME, G6PDH and FAS activities in the muscle.

Adipose tissues and liver from rabbits fed the linseed diet had 15% to 22% less SCD activity than rabbits fed the control diet (Table 5). The muscle enzyme activity was not affected by the diet.

In the LD, the linseed diet increased ( $P < 0.04$ ) the activity of  $\beta$ -HAD (Table 5).

#### Fatty acid composition

In muscle and liver, the linseed diet decreased MUFA proportion and increased PUFA percentages (Table 6). The SFA proportion was not affected by the diet, in both tissues.

There was an increase in the level of EPA, DPA and DHA in both tissues, with the linseed diet, but the arachidonic acid (ARA) percentage was not affected by the diet in both tissues. The proportions of LA and ALA were much higher in rabbits fed the linseed diet than in rabbits fed the control diet, in both tissues. The linseed diet decreased significantly the LA/ALA ratio in the LD, but not in the liver.

**Table 4** Effects of diets (n = 6 per diet) on ME, G6PDH, FAS activities in liver, adipose tissues and the LD

	Control	Linseed	r.s.d.	Diet effect
Unit/g of tissue				
Liver				
ME	2.05	0.61	0.43	**
G6PDH	13.16	10.01	2.20	*
FAS	183.2	108.1	38.85	**
Perirenal fat				
ME	0.65	0.36	0.22	*
G6PDH	8.23	6.38	1.04	**
FAS	106.40	81.42	14.08	*
Interscapular fat				
ME	0.69	0.51	0.14	*
G6PDH	5.21	4.41	0.62	*
FAS	79.30	61.24	13.53	*
LD				
ME	0.59	0.86	0.42	ns
G6PDH	0.22	0.16	0.07	ns
FAS	4.05	4.38	0.89	ns
Unit per entire tissue				
Liver				
ME	149.60	34.81	31.00	***
G6PDH	960.70	570.62	272.31	*
FAS	13 376	6192	4673	**
Perirenal fat				
ME	11.01	5.76	3.10	**
G6PDH	139.93	102.12	28.00	*
FAS	1809	1303	484	*
Interscapular fat				
ME	8.28	6.12	2.20	*
G6PDH	62.52	52.92	9.51	*
FAS	951.60	734.81	185.22	*

ME = malic enzyme; G6PDH = glucose-6-phosphate-dehydrogenase; FAS = fatty acid synthase; LD = *Longissimus dorsi* muscle; ns = no significant difference ( $P > 0.05$ ).

\* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.01$ .

ME, G6PDH, FAS activities are expressed in  $\mu\text{mol NADPH}/\text{min per g of tissue}$ .

**Table 5** Effect of diet (n = 6 per diet) on SCD activity in liver, adipose tissues and LD and on ( $\beta$ -HCoAD activity in the LD

	Control	Linseed	r.s.d.	Diet effect
SCD				
Liver	6.32	4.91	0.82	**
Perirenal fat	26.50	22.62	2.24	***
Interscapular fat	18.71	15.31	2.10	**
LD	0.82	0.69	0.18	ns
$\beta$ -HCoAD				
LD	2.85	3.71	0.71	*

SCD = stearoyl-CoA-desaturase; LD = *Longissimus dorsi* muscle;  $\beta$ -HCoAD =  $\beta$ -hydroxyacyl-CoA-dehydrogenase; ns = no significant difference ( $P > 0.05$ ). SCD activity is expressed in nmol of oleic acid formed/min per g of tissue.  $\beta$ -HCoAD activity is expressed in  $\mu$ mol of disappeared NADH/min per g of tissue. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

The MUFA proportion was lower and the PUFA level was higher in perirenal and interscapular fats of animals fed the linseed diet (Table 7). The SFA proportion was not affected by the diet. There was an increase in the level of EPA, DPA and DHA, with the linseed diet in both tissues. The percentage of ARA was not affected by the diet in the perirenal fat, but it was decreased by the linseed diet in the interscapular fat. The n-6 and n-3 FA proportions were increased by the linseed diet and the LA/ALA was decreased by the experimental diet in both adipose tissues.

The FA composition of polar or non-polar lipids in the LD is summarised in Table 8. The diet significantly affected the amount of different FA. In polar and non-polar fractions, the percentage of all n-3 FA increased in rabbits fed the linseed diet, whereas the MUFA percentage was lower and the SFA percentage was higher.

**Table 6** FA composition in the LD and liver (n = 6 per diet)

Diet	LD				Liver			
	Control	Linseed	r.s.d.	Diet effect	Control	Linseed	r.s.d.	Diet effect
$\Sigma$ SFA	33.17	32.38	1.95	ns	37.40	38.99	1.68	ns
$\Sigma$ MUFA	37.10	27.54	1.05	***	20.79	17.47	1.79	***
$\Sigma$ PUFA	29.73	40.08	1.86	***	41.81	43.74	1.58	*
C18:2n-6	18.00	22.78	1.29	***	24.23	28.04	2.21	**
C20:4n-6	0.31	0.28	0.04	ns	6.29	4.28	2.5	ns
C18:3n-3	9.41	14.95	0.47	***	5.53	6.70	1.01	*
C20:5n-3	0.25	0.30	0.04	*	0.95	1.34	0.22	*
C22:5n-3	0.76	0.85	0.07	*	1.23	1.33	0.08	*
C22:6n-3	0.19	0.26	0.07	*	0.69	0.75	0.11	*
$\Sigma$ n-6	18.48	23.25	1.33	***	32.53	33.44	1.66	ns
$\Sigma$ n-3	11.03	16.57	0.59	***	8.80	10.12	1.12	*
LA/ALA	1.91	1.52	0.06	***	4.40	4.19	0.64	ns

FA = fatty acid; LD = *Longissimus dorsi* muscle; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ns = no significant difference ( $P > 0.05$ ); LA/ALA = linoleic acid/ $\alpha$ -linolenic acid ratio. \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

Results are expressed in percentage of total FA content.

**Table 7** FA composition in interscapular and perirenal fat (n = 6 per diet)

Diet	Interscapular				Perirenal			
	Control	Linseed	r.s.d.	Diet effect	Control	Linseed	r.s.d.	Diet effect
$\Sigma$ SFA	32.61	33.67	2.41	ns	32.16	31.02	2.87	ns
$\Sigma$ MUFA	38.16	26.53	1.55	***	39.26	27.65	1.88	***
$\Sigma$ PUFA	29.23	39.80	1.56	***	28.58	41.33	1.85	***
C18:2n-6	17.35	22.66	0.84	***	17.23	23.76	1.35	***
C20:4n-6	0.48	0.15	0.19	**	0.32	0.27	0.08	ns
C18:3n-3	10.05	15.23	0.66	***	9.86	16.07	0.85	***
C20:5n-3	0.20	0.29	0.07	*	0.14	0.17	0.02	*
C22:5n-3	0.21	0.34	0.07	**	0.23	0.19	0.03	*
C22:6n-3	0.04	0.08	0.03	*	0.03	0.06	0.02	**
$\Sigma$ n-6 FA	18.04	23.16	0.84	***	17.79	24.23	1.31	***
$\Sigma$ n-3 FA	10.94	16.38	0.87	***	10.57	16.88	0.75	***
LA/ALA	1.73	1.49	0.09	***	1.76	1.48	0.09	**

FA = fatty acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; ns = no significant difference ( $P > 0.05$ ); LA/ALA = linoleic acid/ $\alpha$ -linolenic acid ratio.

\* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

Results are expressed in percentage of total FA content.

**Table 8** Polar and non-polar FA composition of the LD (n = 6 per diet)

Diet	Polar lipids				Non-polar lipids			
	Control	Linseed	r.s.d.	Diet effect	Control	Linseed	r.s.d.	Diet effect
ΣSFA	31.30	32.32	0.36	**	31.87	33.02	1.29	*
ΣMUFA	26.65	23.55	0.58	***	39.92	26.95	1.27	***
ΣPUFA	42.05	44.13	0.88	**	28.21	40.03	2.46	***
C18:2n-6	21.42	22.05	1.15	ns	15.91	21.75	1.25	***
C20:4n-6	8.72	8.43	0.65	ns	1.01	1.23	0.23	ns
C18:3n-3	1.93	2.44	0.3	*	9.53	14.62	0.62	***
C20:5n-3	1.30	1.50	0.09	**	0.32	0.48	0.12	*
C22:5n-3	4.01	5.11	0.35	***	0.61	0.93	0.23	*
C22:6n-3	0.89	1.14	0.16	*	0.11	0.18	0.05	*
Σn-6	33.24	33.26	0.91	ns	16.61	22.58	1.31	***
Σn-3	8.56	10.60	0.37	***	11.20	17.05	0.98	***
LA/ALA	3.90	3.14	0.22	**	1.48	1.33	0.04	**

FA = fatty acid; LD = *Longissimus dorsi* muscle; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; LA/ALA = linoleic acid/ $\alpha$ -linolenic acid ratio; ns = no significant difference ( $P > 0.05$ ).

\* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

Results are expressed in percentage of total FA content.

In polar and non-polar lipids, the LA/ALA was decreased by the linseed diet.

#### Lipid oxidation

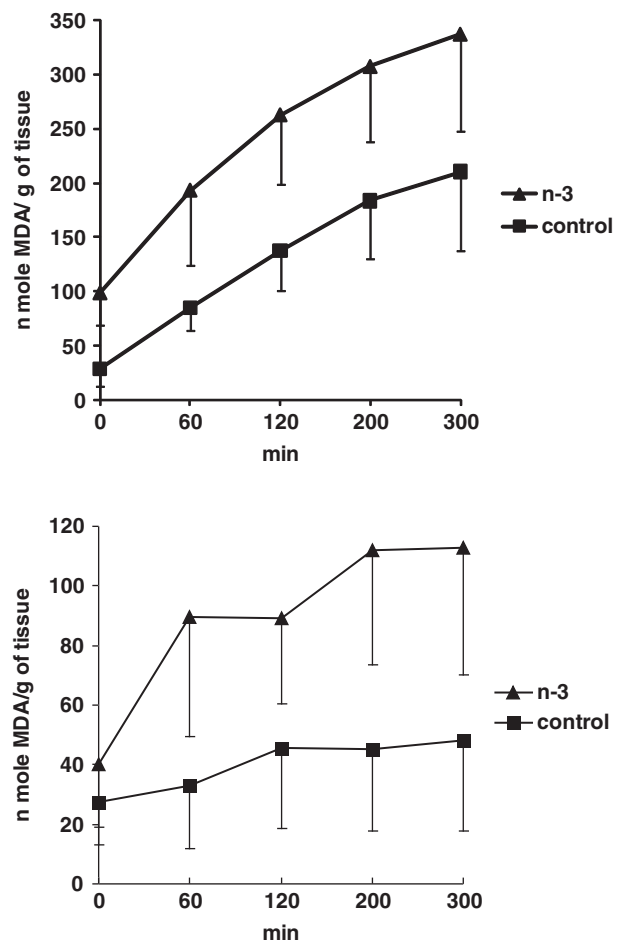
Lipid oxidation was significantly higher in the perirenal fat and the LD of rabbits fed the linseed diet than rabbits fed the control diet ( $P < 0.001$ ; Figure 1).

#### Discussion

This study showed that the incorporation of n-3 FA in the diet had no significant effect on performances and carcass characteristics. These results were in agreement with previous work in rabbits (Fernandez and Fraga, 1996; Combes, 2004; Kouba *et al.*, 2008) and in pigs (Kouba *et al.*, 2003; Guillevic *et al.*, 2009).

The muscular ultimate pH and the lightness ( $b^*$ ) value were not affected by the diet, as already demonstrated by Kouba *et al.* (2008).

ME and glucose-6-phosphate dehydrogenase are the main enzymes involved in supplying NADPH for the reductive biosynthesis of FAs (Wise and Ball, 1964; Young *et al.*, 1964), and FAS is a multi-enzyme that plays a key role in FA synthesis. The hepatic activities of these lipogenic enzymes were much higher than the activities in adipose tissues and muscle. These results are in general agreement with other studies showing that, at 11 weeks of age (age of slaughtering of commercial rabbits), liver is the major site of lipogenesis (60% of total lipogenesis), with adipose tissue accounting for 35% and digestive tract for 5%. (Gondret *et al.*, 1997; Gondret, 1999). Our results also confirmed previous studies showing that, in rabbits, *de novo* lipogenesis is greater in perirenal adipose than in subcutaneous and interscapular adipose tissues (Gondret *et al.*, 1997). When both adipose tissues were considered, the lipogenesis was also lower in the whole interscapular fat than in the whole perirenal fat with the linseed diet.



**Figure 1** Effect of diet on the lipid oxidation (n mole malonaldehyde (MDA)/g of fresh tissue) in perirenal fat (top figure) and in Longissimus dorsi muscle (bottom figure).

The supplementation of n-3 PUFA in the diet decreased lipogenic enzyme activities in the liver and in perirenal and interscapular fats. The consequence of the lower lipogenesis

with linseed diet was that the total lipid depots in these tissues were also reduced. However, linseed diet had no effect on these enzyme activities in the LD. These results were in agreement with a previous study showing that PUFA inhibit hepatic lipogenesis in rabbit (Corino *et al.*, 2002).

SCD generates MUFA from SFA (Bloomfield and Bloch, 1960; Marsh and James, 1962; Gelhorn and Benjamin, 1965). To our knowledge, for the first time this study provides findings about SCD activity in the rabbit. This study also shows that this activity is quite low in rabbit liver, as compared to that of chicken liver, but it is quite similar to that of turkey liver (Kouba *et al.*, 1993) and it is higher than that in pig liver (Kouba *et al.*, 1997). As for lipogenic enzymes, the SCD activity is the highest in perirenal fat, regardless of the diet. The inhibitory effect of PUFA on SCD activity observed in this study in rabbit liver and adipose tissues has already been demonstrated in pig adipose tissue (Kouba and Mourot, 1998; Kouba *et al.*, 2003) and more recently in the muscle of beef cattle (Waters *et al.*, 2009). The lack of effect of linseed diet on muscle enzyme activity in the rabbits observed in this study had also been observed in pig muscle (Kouba *et al.*, 2003). The decrease of the  $\Delta 9$ -desaturase activity in the liver and the adipose tissues of rabbits fed linseed diet compared with those fed the control diet resulted in an obvious reduction in the MUFA percentage, which is consistent with previous studies in the rabbit (Kouba *et al.*, 2008) and in the pig (Kouba *et al.*, 2003). Results of this study showed that the changes in the MUFA percentage could be attributed, at least in part, to a reduction in  $\Delta 9$ -desaturase activity.

In contrast,  $\beta$ -oxidation, estimated by the  $\beta$ -HAD activity, increased in the muscle of animals fed the n-3 PUFA-supplemented diet. Previous studies have already shown that the enhancement of  $\beta$ -oxidation in adipose tissues of mice fed a diet supplemented in EPA and DHA (Flachs *et al.*, 2005). It is well known that PUFA increase transcription of the regulatory  $\mu$ m of FA oxidation, via activation of peroxysome proliferator-activated receptor (PPAR) (Mori *et al.*, 2007).

Our results show that the enrichment of the rabbit diet with extruded linseeds, a major source of n-3 PUFA, led to an enrichment of muscle and adipose tissue n-3 PUFA content, and confirmed previous studies in rabbits (Bernardini *et al.*, 1999; Dal Bosco *et al.*, 2004; Combes and Cauquil, 2006; Petracci *et al.*, 2009). This conclusion has also been demonstrated in pigs (Enser *et al.*, 2000) and in chickens (Crespo and Esteve-García, 2002).

Our results show more favourable PUFA/SFA and LA/ALA ratios in the muscle and adipose tissues from rabbits fed linseed diet, but not in the liver in which diet had no effect on these ratios. These favourable parameters were already demonstrated in a previous work (Kouba *et al.*, 2008).

Lipid oxidation in meat, particularly in rabbits, is mainly due to oxidation of PUFA. Indeed, FA composition of rabbit meat is characterised by a high PUFA content. Therefore, feeding these animals with a linseed diet leads to an increase in the level of n-3 PUFA, which could explain the high value of TBARS registered in this study in muscle and fat.

This tendency agreed with results obtained by Kouba *et al.* (2008), and more recently by Petracci *et al.*, (2009). Our results show a higher risk of peroxidation of the fat compared with the muscle, which is due to a much higher content of lipids, especially PUFA, in perirenal fat. This result has already been shown on Guizhou minipigs (Yang *et al.*, 2010). It is well known that extra supplementation of vitamin E in the diet improves the oxidative stability of the meat and leads to an increase of this vitamin in rabbit meat (Dal Bosco *et al.*, 2004). It seems that the quantity of vitamin E added to the diets in our study (30 mg/kg) was insufficient to limit the deterioration of meat. However, our results are not in agreement with Dal Bosco *et al.* (2004). These authors found that feeding an n-3 PUFA-enriched diet significantly lowered the TBARS level in the LD, but they used a large amount of vitamin E in their study (74 or 289 mg/kg).

## Conclusion

The results of this study show that performances of rabbits are not affected by diet enriched with n-3 PUFA. The PUFA/SFA and LA/ALA ratios were more favourable with an n-3 PUFA enrichment of rabbit diet. Inclusion of linseed in rabbit diet is a valid means of meeting consumer demand for meat that is nutritionally beneficial.

The results of this study suggest that  $\Delta 9$ -desaturase may be involved in the regulation of MUFA content and hence in the quality of rabbit adipose tissue. A better understanding of this enzyme could offer a way to control meat quality in rabbits.

## Acknowledgements

The authors thank Bretagne Lapins for its financial support.

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