World Rabbit Science
World Rabbit Sci. 2006, 14: 247 - 258

EFFECTS OF DIETARY INCLUSION OF DEHYDRATED LUCERNE AND WHOLE LINSEED ON RABBIT MEAT QUALITY

Bianchi M., Petracci M., Cavani C.

Dpt. of Food Science, Alma Mater Studiorum, University of Bologna, P.zza Goidanich 60, 47023 Cesena, Italy

Abstract: The effect of dietary use of dehydrated lucerne meal (DLM) and whole linseed (LIN) on chemical-physical traits, fatty acid composition, susceptibility to lipid oxidation, and sensory quality of rabbit meat and meat products (hamburgers) was studied. Two groups of 128 weaned rabbits (37 d-old) were fed on diets containing 25 or 35% DLM. At 64 d of age, the rabbits were randomly divided into four groups; two of them received the same diet whereas the others were fed on diets containing both 25 or 35% DLM and 8% linseed until slaughtering (87 d). The main effects of DLM (25 *vs.* 35%) and LIN (0 *vs.* 8%) were considered according to a 2×2 factorial statistical design. With regard to the effect of lucerne, the main result was the higher content of α-linolenic acid in meat from rabbits fed the higher level of DLM (6.34 *vs.* 5.82% in DLM35 and DLM25, respectively; $P<0.05$) without impairing the lipid susceptibility to oxidation. The use of linseed strongly ($P<0.01$) influenced the fatty acid composition of the meat by enhancing the content of polyunsaturated fatty acids (PUFA, 33.68 *vs.* 27.79%; for LIN8 and LIN0, respectively) and mainly α-linolenic acid which was three times higher in LIN8 rabbits (9.42 *vs.* 2.95%; $P<0.01$), producing a lower n-6/n-3 PUFA ratio (2.28 *vs.* 6.59; $P<0.01$). However, the higher level of PUFA was related to a higher susceptibility to lipid oxidation (TBARS) of both meat and frozen (-20°C for 3 or 6 months) meat batters for hamburgers production. Despite the higher TBARS, sensory differences among hamburgers were detected only at six months frozen storage. Finally, the use of linseed did not determine a higher colour variation of the packaged hamburgers during storage (14 d at 2-4°C). This experiment indicates a very quick and significant rise in the α-linolenic acid content (three times higher after 3 weeks) when rabbits were fed during the finishing period with a diet containing 8% linseed. However, despite the nutritional importance of this essential fatty acid, care must be taken to avoid lipid oxidation and detrimental effects on the sensory qualities of meat and meat products during processing and subsequent storage.

Key words: rabbit, lucerne, linseed, meat quality, fatty acid composition.

INTRODUCTION

Because of its low contents of fat and cholesterol as well as the high proportion of polyunsaturated fatty acid (PUFA), rabbit meat is generally considered a "healthy" meat (Ouhayoun, 1992; Dalle Zotte, 2002; Combes, 2004). However, its consumption is sometimes rejected because its preparation is considered time-consuming (long cooking) and requires culinary skills. In order to promote the consumption of rabbit meat, some processing plants are trying to develop ready-to-cook and ready-to-eat products. A possible way to improve rabbit meat utilization for convenience foods preparation could be represented by the frozen storage of the meat or meat batters (minced meat with other additives) produced when the price of the meat is lower (during summer time) and used to prepare further processed products when the price of the meat is higher.

For many years, lucerne has been a traditional ingredient in rabbit feeds and represents the source of fibre most widely used in rabbits diets, accounting for about 25-35% of commercial feeds (de Blas and Mateos, 1998).

Correspondence: C. Cavani, claudio.cavani@unibo.it
Received *May 2006* - Accepted *June 2006*.

The dietary use of linseed in animal feeds has been proposed by many authors as a vegetable alternative to fish oil or fish meal, to raise the content of n-3 polyunsaturated fatty acids (n-3 PUFA), and mainly α-linolenic acid (C18:3 n-3) in poultry (Ajuyah *et al.*, 1993), pork (Mattews *et al.*, 2000; Rey *et al.*, 2001; Wood *et al.*, 2003) and rabbit meat (Bernardini *et al.*, 1999; Dal Bosco *et al.*, 2004; Colin *et al.*, 2005). Some authors also reported beneficial effects of linseed on performance, milk composition and viability of the progeny in rabbit does. The general consensus is that providing increased amounts of n-3 essential fatty acids in human nutrition through normal meat consumption can contribute to correct the unbalanced n-6/n-3 PUFA ratio of today's consumer diet and may help to prevent some correlated disease such as hypercholesterolemia-related heart attack and strokes (Prasad, 1997; Simopoulos, 2000; Wood *et al.*, 2003).

Fatty acids are involved in many technological aspects of meat quality. The main problem associated with the modification of the natural fatty acid profile of muscle foods is determined by the ability of unsaturated fatty acids, especially those with more than two double bounds, to oxidise and reduce the shelf-life of meat products (Wood *et al.*, 2003). This problem could also be more serious when the meat with a high level of PUFA is used for further processing that involves mincing, long term frozen storage, and cooking. In fact it has been widely reported that lipid oxidation represents a key-role in the development of cooked meat flavour (Enser, 1999). In pigs, it has been suggested that dietary use of linseed could impair the flavour of cooked meat when the α-linolenic (C18:3 n-3) acid content of the meat is above 3% of total fatty acids (Campo *et al.*, 2003; Wood *et al.*, 2003). Another technological problem of meat and meat products with a high susceptibility to lipid oxidation is represented by the colour variation over storage, because of the oxidation of red oxymyoglobin to brown metmyoglobin in parallel with the lipid oxidation reaction, with detrimental effects on product appearance (Jensen *et al.*, 1998).

The aim of this study was to investigate the effect of dietary use of dehydrated lucerne meal (DLM 25 *vs.* 35%) and linseed (LIN 0 *vs.* 8%) in growing rabbits on chemical-physical traits, fatty acid composition, susceptibility to lipid oxidation, and sensory quality of meat and meat products (hamburgers).

MATERIAL AND METHODS

Animals and diets

A total of 256 weaned rabbits (37 days-old) belonging to the hybrid strain TOP 97® (Martini and C. S.p.A., Italy) were divided into two groups and fed *ad libitum* on diets containing 25 or 35% respectively of dehydrated lucerne meal (DLM25LIN0; DLM35LIN0) until 64 days-old. The diets used during the first growing period (37-64 days-old) were medicated; medication treatment was represented by the addition of zinc bacitracin at a level of 150 mg/kg feed. At 64 d, the rabbits were divided into four sub-groups. Two of them were fed on the same diets (DLM25LIN0; DLM35LIN0, without medication), whereas the other groups were fed on diets containing both 25 or 35% DLM and 8% whole linseed (DLM25LIN8; DLM35LIN8) until slaughter age (87 d). The rabbits were housed in pairs under intensive conditions. The ingredients and chemical composition of the diets are reported in Table 1. The diets were isoproteic and their composition mainly differed in the amount of dehydrated lucerne meal and whole linseed. The substitution of 10% DLM was obtained by using soy hulls (6%) and sunflower meal (4%) whereas the substitution of 8% linseed was by sunflower meal (4.8%) and wheat (3.2%). Finally, in order to improve product quality and shelf life, α-tocopheryl acetate (vit. E) was added to all diets at an inclusion rate of 200 mg/kg.

Productive performances and carcass traits determination

In accordance with the experimental design, the influence of the diet on productive performance of rabbits was separately determined during the first (37-64 d), and second (65-87 d) growing period by

Table 1: Ingredients and chemical composition of the diets.

	Diet			
	DLM25LIN0	DLM25LIN8	DLM35LIN0	DLM35LIN8
Ingredients (%)				
Dehydrated lucerne meal	25	25	35	35
Whole linseed	-	8	-	8
Wheat bran	25.6	25.6	25.1	25.1
Sunflower meal	17	12.2	13	8.2
Barley grain	11	11	11	11
Wheat grain	6	2.8	6	2.8
Soy hulls	8	8	2	2
Soy bean meal	2	2	2.5	2.5
Beet molasses	2.5	2.5	2.5	2.5
Calcium carbonate	1.6	1.6	1.6	1.6
Calcium diphosphate	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Vitamin-mineral premix	0.3	0.3	0.3	0.3
α-tocopheryl-acetate	0.02	0.02	0.02	0.02
Chemical composition (%)				
Digestible energy (MJ/kg)[1]	9.7	10.3	9.8	10.4
Crude protein	17.1	17.1	17.3	17.3
Ether extract	2.9	5.5	3.1	5.7
Crude Fibre	16.0	15.3	15.6	15.0
Main fatty acids (%)				
Linoleic acid (C18:2 n-6)	49.2	34.0	48.4	33.8
α-linolenic acid (C18:3 n-3)	10.1	29.7	11.0	30.2
PUFA n-6/n-3	4.9	1.1	4.4	1.1

[1] DE calculated according to the equation reported by Maertens *et al.*, (1988)

measuring body weights and feed intakes at 37, 64 and 87 days-old (n=64 rabbits per group). Body weight was individually measured whereas feed intake was determined in four sub-groups of 16 rabbits per treatment. The daily weight gain (DWG, g/d) and feed conversion ratio (FCR, g/d) were subsequently calculated. The rabbits were slaughtered at 87 days-old under commercial conditions and carcasses prepared as recommended by Blasco and Ouhayoun (1996).

The chilled carcass weight (after 24 h at 0-2°C) and dressing out percentage were determined by considering the overall slaughtered rabbits (n=229), whereas the loin and hind part percentages; perirenal, scapular, inguinal and total dissectible fat were determined on 12 carcasses per group and expressed as percentage of the chilled carcass (Blasco and Ouhayoun, 1996).

Meat quality evaluation

Twelve carcasses from each group were randomly collected and used for meat quality analyses at 24 h *post mortem* (2-4°C). The *L. lumborum* muscles (between the 1st and 7th lumbar vertebra) were used to determine colour, pH, water holding capacity (drip loss, released water by filter paper press method,

and cooking loss) whereas the hind leg muscles were analyzed for total lipid content, fatty acid composition and lipid susceptibility to oxidation according to the methods described below.

In order to prepare rabbit meat hamburgers, 35 carcasses from each group were separately boned and the dissected meat (about 30 kg per group) was used to prepare, under commercial conditions, four different meat batters (DLM25LIN0; DLM35LIN0; DLM25LIN8; DLM35LIN8) by mixing the minced meat in a paddle meat mixer with water (63 g/kg of meat), sodium chloride (14 g/kg) and a commercial seasoning (ingredients: spices, sodium chloride, glucose, lactose, sucrose and ascorbic acid) (23 g/kg). Thirty-two hamburgers (n=8 per group) (70 g of weight, 8.5 cm diameter and 1.5 cm thickness) were subsequently prepared and analyzed for colour, pH and cooking loss. Furthermore, 8 hamburgers per group were packaged under normal atmospheric conditions and used to determine the colour variation during 14 days storage at 2-4°C. Finally, 20 kg of meat batter belonging to each group were vacuum packaged, and stored at –20°C for 3 or 6 months before measuring lipid susceptibility to oxidation and carrying out a triangular sensory test.

Colour determination

The colour parameters L* (lightness), a* (redness), and b* (yellowness) (CIE, 1976) were determined by using a Minolta CR-300 Chroma Meter operating with light source C. The colour of *L. lumborum* was measured on the epymisial surface of the muscle, whereas the colour of hamburgers was determined by averaging three colour measurements taken on the surface of each hamburger. In order to evaluate the colour changes of the packaged hamburgers, colour readings were taken over 14 days storage at 2-4°C and used to calculate hue (h, $\tan^{-1}$ b*/a*), saturation (C*, $\sqrt{(a^*)^2+(b^*)^2}$) and colour difference over time ($\Delta E^*=[(L_1^*-L_2^*)^2+(a_1^*-a_2^*)^2+(b_1^*-b_2^*)^2]^{1/2}$; where: L_1^*, a_1^*, b_1^* = initial colour readings at time "0", and L_2^*, a_2^*, b_2^* = colour readings at day 3, 5, 7, 10, 12 and 14).

pH and water holding capacity determination

The pH at 24 hours *post mortem* was measured by using the direct probe-method and a portable pH-meter (mod. HI98240, Hanna Inst.) equipped with a glass electrode (mod. FC230, Hanna Inst.). The water holding capacity (WHC) was assessed according to drip loss (*L. lumborum*), released water by filter paper press method (*L. lumborum*), and cooking loss (*L. lumborum* and hamburgers). Drip loss was determined by calculating the loss (%) of water from a whole boned *L. lumborum* muscles kept suspended in glass box for 24 h at 2-4°C (Honikel, 1998). Released water was determined by filter paper press method and carried out on *L. lumborum* muscles according to the procedure described by Pla and Apolinar (2000). Meat slices of 300±5 mg were removed from the muscle and placed on a previously weighed filter-paper Whatman No 1. The paper with the meat slice was subsequently placed between two plexiglas plates and a load of 1 kg was applied for 5 min. Damp filter paper was rapidly weighed after accurately removing the compressed meat. The mean of three replicates was considered as a value. WHC was expressed as a percentage of released water.

Cooking loss on *L. lumborum* was measured on a whole boned muscle from each carcass. The raw samples were individually weighed, vacuum packaged in a plastic bag and cooked in a water bath at 80°C. The cooking time was stopped when reaching 75°C (monitored with a food thermocouple) at core sample. The samples were then removed from the water bath, cooled under tap water and reweighed. Cooking loss was expressed as percentage of the initial sample weight (Honikel, 1998). Cooking loss in hamburgers was determined by cooking the hamburgers in a conventional oven at 180°C until reaching 75°C (monitored with a food thermocouple) at core sample. The samples were then allowed to cool to room temperature and reweighed. Cooking loss was expressed as a percentage of the initial sample weight.

Lipid content, fatty acid composition and susceptibility to lipid oxidation
The total lipid content was determined by a pressurized solvent extraction method by using an Accelerated Solvent Extraction Automatic System (ASE 200, Dionex, Salt Lake City, Utah, U.S.A.) and a chloroform/methanol (2:1) extracting solution according to the procedure reported by Toschi *et al.* (2003). The fatty acid composition of total lipid was determined by gas-liquid chromatography. The fatty acid methyl esters were prepared by KOH/met-OH transesterification and analysed on a CP-Sil 88 capillary column (50 m × 0.25 mm internal diameter) (Chrompack, UK, Ltd, London). Peaks were identified using standards (Nu-Check-Prep. Inc., Elysian, MN, USA) and results expressed as percentage by weight of total fatty acids methyl esters.

The unsaturation index (Bordoni *et al.*, 1999) was calculated as follows:

$$\sum_{1}^{n}{}_{i} \; \%\, fatty\ acid_i \cdot number\ of\ double\ bounds\ in\ the\ fatty\ acid_i$$

The susceptibility of muscle tissue homogenates to iron-induced lipid oxidation was determined according to the method of Kornbrust and Mavis (1980). Homogenates were incubated at 37°C and aliquots were removed at fixed time intervals (0, 30, 60, 90 and 150 min) for measurement of 2-thiobarbituric acid-reactive substances (TBARS). Protein content of the meat was determined according to the Lowry procedure (Lowry *et al.*, 1951) and TBARS expressed as nmoles malonaldehyde (MDA)/mg protein.

Sensory analysis
A triangular sensory test was performed in order to detect the existence of sensory differences between the frozen meat batters stored (-20°C) for 3 or 6 months. Two treatments (DLM35LIN0 *vs.* DLM35LIN8) were compared during the test execution.

The samples (hamburgers) for sensory analysis were prepared with thawed (24 h at 2-4°C) meat batters and cooked in a conventional oven at 180°C until reaching 75°C at core. The samples were served warm, according to a randomised complete block design to 18 untrained judges. Each judge was presented with three samples (i.e. DLM35LIN0, DLM35LIN0, DLM35LIN8) and asked to discriminate between the different samples. According to test conditions (*a*, probability of saying that a difference occurs when it does not = 0.05; *b*, probability of saying that no difference occurs when it does = 0.20; *Pa*, proportion of panellists able to detect differences between the two tested products = 50%) a minimum of 10 correct responses was established to find a significant (P=0.05) difference between the DLM35LIN0 or DLM35LIN8 samples (Schlich, 1993; Meiligaard *et al.*, 1999).

Statistical analyses
Data were analyzed using a two ways factorial analysis of variance (ANOVA, GLM) (SAS Institute, 1988). The model tested the main effect of inclusion level of dehydrated lucerne meal (25 *vs.* 35%) and linseed (0 *vs.* 8%) as well as the interaction term using residual error.

RESULTS AND DISCUSSION

Productive performances and carcass traits
The overall zootechnical performances were satisfactory and in line with performances of rabbits under commercial conditions. The overall mortality at the end of growing phase was 8.9% and was not related to the type of diet. However, some differences among treatments were observed on feed efficiency and growth. The higher inclusion level of dehydrated lucerne meal (DLM35 *vs.* DLM25) determined a lower feed conversion ratio (3.09 *vs.* 3.30 g/g; P<0.05) during the first growing period (37-64 d) and a lower daily weight gain (34.1 *vs.* 36.6 g/d; P<0.01) during the second growing period (65-87 d). The dietary use of linseed (LIN8 *vs.* LIN0) determined a lower daily weight gain (33.9 *vs.*

36.8 g/d; $P<0.01$) during the second growing period as well as a lower live weight of rabbits at 87 days (2652 *vs.* 2741 g; $P<0.01$).

These results are consistent with Ajuyah *et al.* (1993) who found a slightly lower live weight and poorer weight gain in broiler chickens fed on a diet containing 15% whole linseed when compared with a control diet. These authors associated the poorer growth rate to the presence of toxic substances in raw whole flaxseed which may depress energy utilization. More recently, Colin *et al.* (2005) using diets containing extruded linseed, reported a decreased growth and lower live weight at slaughter in rabbits fed linseed. This evidence was also found by Verdelhan *et al.* (2005) who observed a decreased (-70g) live weight of rabbits at slaughter by using linseed oil in the diet. However, Bernardini *et al.* (1999) and Dal Bosco *et al.* (2004) did not observe any detrimental effect of linseed on productive performances of rabbits.

In Table 2 the slaughter and carcass traits are reported. The level of DLM did not determine any difference in the considered parameters. Whereas the use of linseed produced a lower carcass weight (1522 *vs.* 1585 g; $P<0.01$), which is consistent with the lower live weight at slaughter. However, the dressing out percentage was not impaired by dietary use of linseed. Finally, higher perirenal (1.88 *vs.* 1.53%; $P<0.05$) and total (2.84 *vs.* 2.43%; $P<0.05$) dissectible fats were found in the LIN8 group despite the fact that feed conversion ratio was not affected by linseed.

Meat quality

The chemical-physical traits of *L. lumborum*, hind leg muscles and hamburgers are reported in Table 3. The overall chemical-physical traits were not strongly affected by the diet. With regard to the inclusion level of dehydrated lucerne (DLM25 *vs.* DLM35), a significant effect was only observed on the pH, which was higher ($P<0.01$) in DLM25 (6.24 *vs.* 6.12; and 6.11 *vs.* 6.07 for *L. lumborum* and hamburgers, respectively). Concerning the effect of linseed, the meat (*L. lumborum*) from rabbits fed on diets containing linseed (LIN8), exhibited higher a* values (3.73 *vs.* 2.74; $P<0.01$). Moreover, the hamburgers prepared with the LIN8 meat, exhibited a lower pH and higher L* values ($P<0.01$). No differences among treatments were found in drip loss, released water and cooking loss. Finally, the diet did not influence the lipid content of hind leg muscles.

Table 2: Slaughter and carcass traits.

	Diet[1]					Significance[2]	
	DLM		LIN				
	25	35	0	8	Pooled SEM	DLM	LIN
No.	112	117	113	116			
Chilled carcass weight (g)	1562	1545	1585	1522	11.5	ns	**
Dressing out percentage (%)	58.3	57.9	58.2	58.1	0.30	ns	ns
No.	24	24	24	24			
Loin percentage[3] (%)	24.7	24.3	24.3	24.6	0.22	ns	ns
Hind part percentage[3] (%)	32.9	32.5	33.0	32.4	0.19	ns	ns
Perirenal fat[3] (%)	1.81	1.61	1.53	1.88	0.09	ns	*
Scapular fat[3] (%)	0.52	0.56	0.53	0.55	0.02	ns	ns
Inguinal fat[3] (%)	0.36	0.42	0.37	0.41	0.03	ns	ns
Total dissectible fat[4] (%)	2.69	2.58	2.43	2.84	0.10	ns	*

SEM: standard error of the mean. *P<0.05; **P<0.01; ns: not significant . [1]DLM: dehydrated lucerne meal; LIN: whole linseed. [2]No interaction "DLM*LIN" was found. [3]Calculated on the basis of chilled carcass weight. [4]Sum of perirenal, scapular and inguinal fat deposits.

Table 3: Chemical-physical traits of *L. lumborum*, hind leg muscles and hamburgers.

			Diet[1]					Significance[2]	
			DLM		LIN		Pooled SEM		
			25	35	0	8		DLM	LIN
L. lumborum	No.		24	24	24	24			
	pH24h		6.24	6.12	6.16	6.19	0.03	**	ns
	Drip loss (%)		1.17	1.11	1.14	1.14	0.08	ns	ns
	Released water (%)		14.5	15.4	14.9	14.9	0.49	ns	ns
	Cooking loss (%)		13.8	14.6	14.0	14.4	0.50	ns	ns
	Colour	L*	44.6	45.7	45.6	44.7	0.33	ns	ns
		a*	3.13	3.34	2.74	3.73	0.18	ns	**
		b*	0.31	0.35	0.48	0.17	0.08	ns	ns
Hind leg muscles	No.		16	16	16	16			
	Total lipid (%)		2.98	3.12	2.96	3.14	0.12	ns	ns
Hamburgers	No.		16	16	16	16			
	pH24h		6.11	6.07	6.12	6.05	0.01	**	**
	Cooking loss (%)		15.32	16.25	15.44	16.12	0.32	ns	ns
	Colour	L*	55.41	55.34	54.51	56.23	0.29	ns	**
		a*	13.12	13.00	13.24	12.89	0.17	ns	ns
		b*	7.31	7.30	7.12	7.48	0.27	ns	ns

SEM: standard error of the mean. *P<0.05; **P<0.01; ns: not significant.[1]DLM: dehydrated lucernemeal LIN: whole linseed. [2] No interaction "DLM*LIN" was found.

It should be pointed out that the ultimate pH values of the meat found in this study seem to be slightly higher than normal. This could be due to the effect of a long preslaughter transportation (about 6 hours) of the animals that was necessary because of the distance from the farm to the abattoir (Jolley, 1990; Hulot and Ouhayoun, 1999).

The effect of DLM and LIN on fatty acid composition of hind leg meat is reported in Table 4. The meat from rabbits fed the higher level of lucerne (DLM35) exhibited a higher content of PUFA n-3 (7.22 *vs.* 6.61%; P<0.01), determined by the higher level of α-linolenic acid (C18:3 n-3) (6.34 *vs.* 5.82%; P<0.05) which produced a lower n-6/n-3 PUFA ratio (4.23 *vs.* 4.79; P<0.01). On the other hand, the higher level of lucerne also determined a higher content of saturated fatty acids (Total SFA) (41.15 *vs.* 39.81%; P<0.05) and a lower content of monounsaturated fatty acids (total MUFA) (28.05 *vs.* 30.01 %; P<0.05).

The dietary use of linseed strongly influenced the overall fatty acid composition of the meat determining a lower content of Total SFA (38.10 *vs.* 42.75%; P<0.01) and a higher content of Total PUFA (33.68 *vs.* 27.79%; P<0.01). The latter was due to the higher contents of α-linolenic (9.42 *vs.* 2.95%; P<0.01) and docosapentaenoic (DPA) (C22:5 n-3, 0.37 *vs.* 0.20%; P<0.01). These results determined a more favourable PUFA/SFA (0.89 *vs.* 0.65; P<0.01) and n-6/n-3 PUFA (2.28 *vs.* 6.59; P<0.01) ratios in the meat from rabbit fed diets containing linseed.

The effectiveness of whole linseed in increasing the PUFA and α-linolenic acid contents in the meat has been previously reported by several studies on both rabbit (Bernardini *et al.,* 1999; Dal Bosco *et al.*, 2004) and other species (Mattews *et al.*, 2000; Riley *et al.*, 2000; Rey *et al.*, 2001). Moreover, Colin *et al.* (2005) recently proposed the use of a commercial feed ingredient (Tradi-Lin®) containing extruded

Table 4: Fatty acid composition (% of total methyl ester) of hind leg muscles.

	Diet[1]				Pooled	Significance[2]	
	DLM		LIN				
	25	35	0	8	SEM	DLM	LIN
No.	16	16	16	16			
Fatty acid (%)							
14:0 (myristic)	2.86	3.13	3.17	2.82	0.10	ns	ns
15:0	0.48	0.55	0.56	0.47	0.03	ns	ns
16:0 (palmitic)	28.58	28.97	31.05	26.36	0.48	ns	**
17:0 (margaric)	0.58	0.89	0.61	0.87	0.11	ns	ns
18:0 (stearic)	7.31	7.62	7.37	7.58	0.13	ns	ns
Total SFA	*39.81*	*41.15*	*42.75*	*38.10*	*0.52*	*	**
14:1 (myristoleic)	0.41	0.25	0.35	0.31	0.04	*	ns
15:1	0.16	0.06	0.10	0.12	0.04	ns	ns
16:1 (palmitoleic)	4.51	3.28	4.47	3.24	0.24	**	**
17:1	0.25	0.25	0.30	0.21	0.03	ns	ns
18:1 n-7 (vaccenic)	1.26	1.15	1.27	1.14	0.03	ns	*
18:1 n-9 (oleic)	23.43	23.05	23.09	23.38	0.27	ns	ns
Total MUFA	*30.01*	*28.05*	*29.56*	*28.40*	*0.49*	*	*ns*
18:2 n-6 (linoleic)	21.09	21.50	21.38	21.21	0.22	ns	ns
20:2 n-6	0.19	0.25	0.24	0.20	0.04	ns	ns
20:3 n-6 (dihomo-γ-linolenic)	0.23	0.19	0.27	0.14	0.04	ns	ns
20:4 n-6 (arachidonic)	1.93	1.73	1.97	1.68	0.11	ns	ns
22:4 n-6	0.25	0.08	0.22	0.11	0.04	*	ns
Total PUFA n-6	*23.69*	*23.75*	*24.07*	*23.35*	*0.30*	*ns*	*ns*
18:3 n-3 (α -linolenic)	5.82	6.34	2.95	9.42	0.59	*	**
20:5 n-3 (eicosapentaenoic, EPA)	0.39	0.44	0.46	0.37	0.05	ns	ns
22:5 n-3 (docosapentaenoic, DPA)	0.28	0.27	0.20	0.37	0.03	ns	**
22:6 n-3 (docosahexaenoic, DHA)	0.12	0.16	0.11	0.18	0.05	ns	ns
Total PUFA n-3	*6.61*	*7.22*	*3.72*	*10.33*	*0.60*	**	**
Total PUFA	*30.30*	*30.97*	*27.79*	*33.68*	*0.63*	*ns*	**
PUFA/SFA	0.77	0.76	0.65	0.89	0.02	ns	**
n-6/n-3	4.79	4.23	6.59	2.28	0.40	**	**
Unsaturation index	103.55	102.94	95.15	111.83	1.66	ns	**

SEM: standard error of the mean. *P<0.05; **P<0.01; ns: not significant. [1]DLM: dehydrated lucerne meal, LIN: whole linseed. [2]No interaction "DLM*LIN" was found.

linseed to increase the a-linolenic acid contents of rabbit meat. As regards the lipid susceptibility to oxidation of the fresh leg meat and frozen meat batters, no differences were observed between the two inclusion levels of lucerne (DLM, 25 *vs.* 35%) (Figure 1). On the other hand, the linseed (LIN8) determined a significantly (P<0.01) higher susceptibility to lipid oxidation in both leg meat and meat batters. The higher susceptibility to lipid oxidation can be attributed to the increased content of α-linolenic acid in the meat which was almost three times higher. It is well known that α-linolenic acid plays a key-role in determining the susceptibility to oxidation of the meat (Enser, 1999; Rey *et al.*,

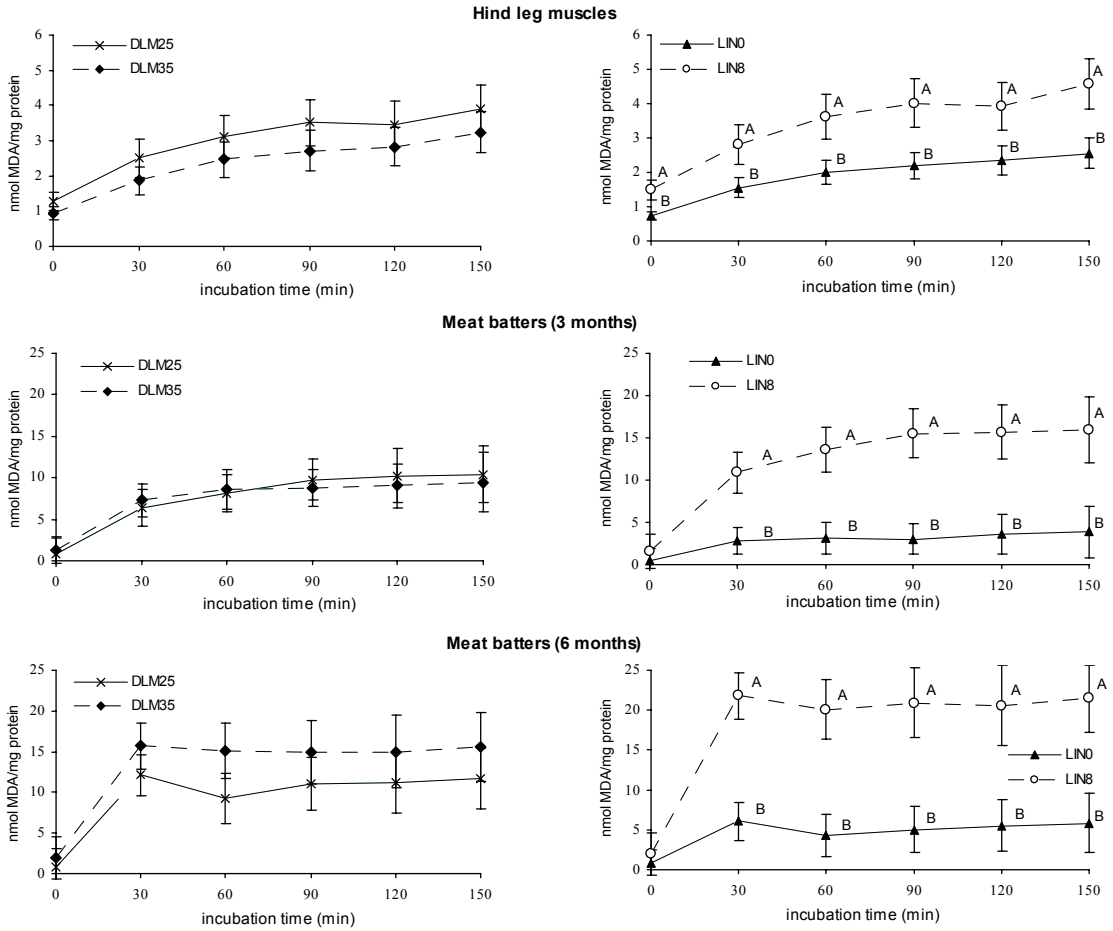

Figure 1: Susceptibility to lipid oxidation (induced TBARS) of hind leg muscles and frozen (3 and 6 months at -20°C) meat batters (n=8 samples per treatment per incubation time. A,B: $P<0.01$).

2001). Dal Bosco *et al.* (2004) observed a positive effect of raised inclusion level of α-tocopheryl-acetate (up to 289 mg/kg) in the diet on the TBARS values of both fresh (24 h *post mortem*) and stored (8 days) meat obtained from rabbits fed on a diet containing the same percentage of linseed adopted in this study (8%).

With regard to sensory analysis, storing the meat batters for three months did not produce any appreciable sensory difference among the treatments (DLM35LIN0 *vs.* DLM35LIN8) (Table 5). These results are consistent with Colin *et al.* (2005) who did not observe any alteration of hedonic characteristics of fresh rabbit meat with a raised content of n-3 PUFA obtained by dietary use of extruded linseed. However, at 6 months of storage, twelve of the eighteen judges ($P=0.05$) who participated in the triangular sensory test were able to differentiate the two types of hamburgers (Table 5). These results indicate that some variation in the sensory properties of the meat (off-flavours, rancidity, etc.) occurred during frozen storage, and suggest that rabbit meat products with raised PUFA should not be stored for long periods. However, in order to promote the use of frozen meat batters for the production of hamburgers or other processed products, further investigations are needed to determine exactly when the modification of sensory properties should be considered unacceptable for consumers.

Table 5: Triangular sensory test of rabbit meat hamburgers prepared with DLM35LIN0 and DLM35LIN8 meat batters frozen stored for 3 and 6 months.

Storage time (months)	No. panellists	No. panellists that did not recognize the different sample	No. panellists that did recognize the different sample	Significance
3	18	14	4	ns
6	18	6	12	*

*P<0.05; ns: not significant.

Figure 2 shows the influence of linseed on colour variation (lightness, L*; hue, H*; saturation, C*; colour difference, ÄE*) over 14 days storage (2-4°C) of packaged hamburger. The major colour changes (ÄE*) of the hamburgers were observed during the first 7 days of storage in both LIN0 and LIN8 groups. However, no important differences in the evolution of colour parameters were found between treatments indicating that raised amounts of PUFA in the meat have no relevant impact on hamburger colour changes during storage. This result represents an advantage for the production of processed meat products and could be due to the supplementation of the diets with 200 mg/kg α-tocopheryl-acetate which is well known to positively impact the colour stability of the meat (Jensen *et al.*, 1998).

In conclusion, the most interesting result has been obtained when considering the effect of linseed on fatty acid composition and susceptibility to lipid oxidation of meat and meat products (hamburgers).

The results demonstrate that the nutritional value of rabbit meat can be improved by increasing itsα-linolenic acid content up to three times through the use of diets containing 8% whole linseed during the last three weeks of the growing phase. However, although the overall quality traits of fresh raw

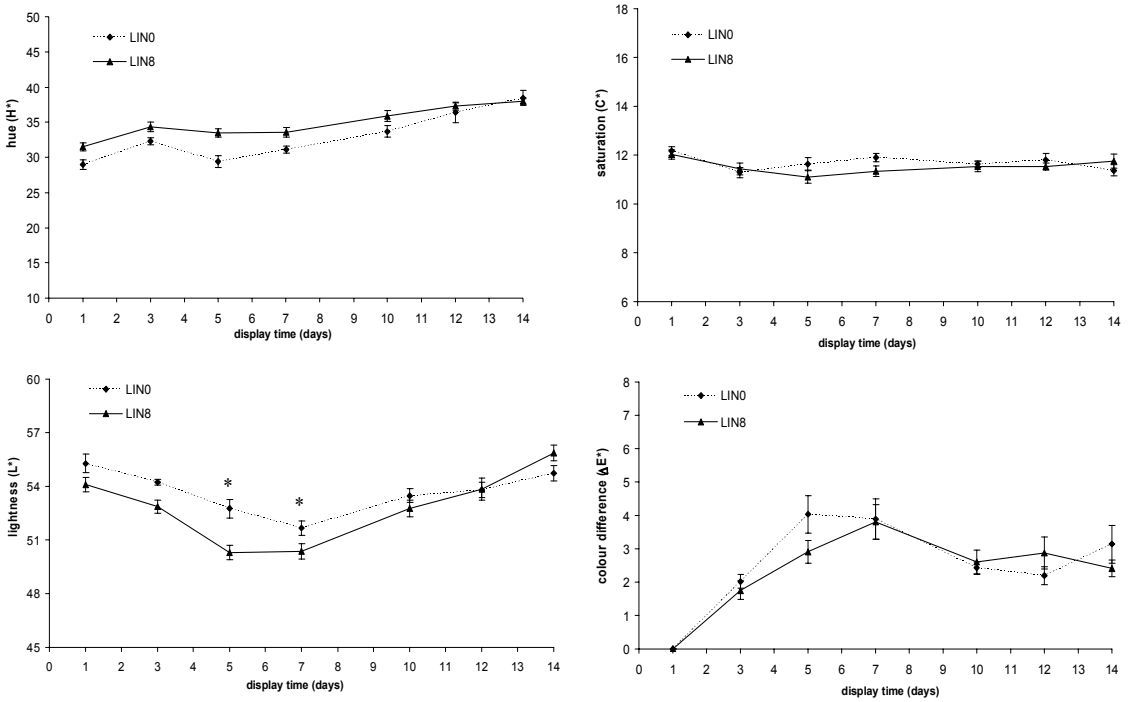

Figure 2: Colour variation (hue, saturation, L*) and colour changes (ÄE*) during storage (14 days at 2-4°C) of packaged rabbit meat hamburgers (n=16 hamburgers per treatment per display time). *P<0.05

meat with increased PUFA n-3 were not modified to a high extent, when this meat is used to prepare further processed products (hamburger-type) which involve mincing, mixing with additives and frozen storage, lipid oxidation and modification of sensory properties of the products may occur.

Acknowledgements: The authors are grateful to "F.lli Martini & C. S.p.A." for technical assistance. Research founded by MIUR - PRIN ex 40%.

REFERENCES

Ajuyah A.O., Fenton T.W., Hardin R.T., Sim J.S. 1993. Effect of dietary full flaxseed with and without antioxidant on the fatty acid composition of major lipid classes of chicken meats. *Poult. Sci., 72, 125-136.*

Bernardini M., Dal Bosco A., Castellini C. 1999. Effect of dietary n-3/n-6 ratio on fatty acid composition of liver, meat and perirenal fat in rabbits. *Anim. Sci., 68, 647-654.*

Blasco A., Ouhayoun J. 1996. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Sci., 4, 93-99.*

Bordoni A., Biagi P.L., Hrelia S. 1999. The impairment of essential fatty acid metabolism as a key factor in doxorubicin-induced damage in cultured rat cardiomyocytes. *Biochim. Biophys. Acta, 1440, 100-106.*

Campo M.M., Nute G.R., Wood J.D., Elmore S.J., Mottram D.S., Enser M. 2003. Modelling the effect of fatty acids in odour development of cooked meat in vitro: part I – sensory perception. *Meat Sci., 3, 367-375.*

CIE 1976. Recommendations on uniform color spaces, color differences, equations. Psychometric color terms. *Supplement No.2 to CIE Publications n.15. Commission Intenationale de l'Eclairage, Colorimetry. Paris, France.*

Colin M., Raguenes N., Le Berre G., Charrier S., Prigent A.Y., Perrin G. 2005. Influence d'un enrichissement de l'aliment en acides gras oméga 3 provenant de graines de lin extrudées (Tradi-Lin®) sur les lipides et les caractéristiques de la viande de lapin. In Proc.: *11èmes Journées de la Recherche Cunicole, Paris, 163-166.*

Combes S. 2004. Valeur nutritionelle de la viande de lapin, INRA, *Prod. Animale, 17, 373-383.*

Dal Bosco A., Castellini C., Bianchi L., Mugnai C. 2004. Effect of dietary α-linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. *Meat Sci., 66, 407-413.*

Dalle Zotte A. 2002. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Liv. Prod. Sci., 75, 11-32.*

De Blas C., Mateos G.C. 1998. Feed Formulation. *In: Blas C., Wiseman J. (ed.). The Nutrition of the Rabbit. CAB International, London, UK, 241-253.*

Enser M. 1999. Nutritional effects on meat flavour and stability. *In: Richardson R.I., Mead G.C. (ed.). Poultry Meat Science. CABI Publishing, Oxfordshire, UK, 197-215.*

Honikel K.O. 1998. Reference methods for the assessment of physical characteristics of meat. *Meat Sci., 49, 447-457.*

Hulot F., Ouhayoun J. 1999. Muscular pH related traits in rabbits: a review. *World Rabbit Sci., 7, 15-36.*

Jensen C., Lauridsen C., Bertelsen G. 1998. Dietary vitamin E: Quality and storage stability of pork and poultry. *Trends Food Sci. Technol., 9, 62-72.*

Jolley 1990. Rabbit transport and ist effect on meat quality. *Applied Animal Behaviour Sci., 28, 119-134.*

Kornbrust D.J., Mavis R.D. 1980. Relative susceptibility of microsomes from lung, heart, liver kidney, brain, tests to lipid peroxidation. *Lipids, 15, 315-322.*

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem., 193, 265-275.*

Maertens L., Moermans R., De Groote G. 1988. Prediction of apparent digestible energy content of commercial pelleted feeds for rabbits. *J. Appl. Rabbit Res.., 11, 60-67.*

Maertens L., Aerts J.M., De Brabander D.L. 1999. Effet d'un aliment riche en acides gras omega-3 sur les performances et la composition du lait des lapines et la viabilité de leur descendance. In Proc.: *11èmes Journées de la Recherche Cunicole, Paris,, 205-208.*

Matthews K.R., Homer D.B., Thies F., Calder P.C. 2000. Effect of whole linseed *Linum usitatissimum* in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues. *Br. J. Nutr., 83, 637-643.*

Meilgaard M., Civille G.V., Carr B.T. 1999. Sensory Evaluation Techniques*, 3rd ed., CRC Press, New York.*

Ouhayoun J. 1992. La viande de lapine. Caracteristiques et variabilite qualitative. *Cuni-Sciences, 7, 1-15.*

Pla M., Apolinar R. 2000. The Filter Paper Press as a method for measuring water holding capacity of rabbit meat. *Word Rabbit Sci., Valencia ,Jul. 2000, vol. A, 659-662.*

Prasad K. 1997. Dietary flax seed in prevention of hypercolesterolemic atherosclerosis. *Atherosclerosis, 132, 69-76.*

Rey A.I., Kerry J.P., Lynch P.B., Lopez-Bote C.J., Buckley D.J., Morrissey P.A. 2001. Effect of dietary oils and alpha-tocopheryl acetate supplementation on lipid TBARS and cholesterol oxidation in cooked pork. *Anim. Sci., 72, 457-463.*

Riley P.A., Enser M., Nute G.R., Wood J.D. 2000. Effects of dietary linseed on nutritional value and other quality aspects of pig muscle and adipose tissue. *Anim. Sci., 71, 483-500.*

SAS Institute 1988. SAS®/STAT Guide for Personal Computers. Version 6.03 Edition. *SAS Institute Inc., Cary, NC.*

Schlich P. 1993. Risk tables for discrimination tests. *Food Qual. Preference, 4, 141-151.*

Simopoulos, A.P. 2000. Human requirement for n-3 polyunsaturated fatty acids. *Poult. Sci., 79, 961-970.*

Toschi T.G., Bendini A., Ricci A., Lercker G. 2003. Pressurized solvent extraction of total lipids in poultry meat. *Food Chem., 83, 551-555.*

Verdelhan S., Bourdillon A., Renouf B., Audoin E. 2005. Effet de l'incorporation de 2% d'hulie de lin dans l'aliment sur les performances zootechniques et sanitaries de lapins en croissance. In Proc.*: 11èmes Journées de la Recherche Cunicole, Paris,, 209-211.*

Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., Sheard P.R., Enser M. 2003. Effects of fatty acids on meat quality: a review. *Meat Sci., 1, 21-32.*