EFFECT OF ENRICHED DIETS ON FAT COMPOSITION, SENSORY CHARACTERISTICS AND NUTRITIONAL VALUE OF RABBIT MEAT

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ABSTRACT

The aim of this study was to evaluate the effect of modifying the diet of rabbits on the intramuscular fat composition through the addition of different oils (high oleic sunflower, fish), oilseeds (flax, chia, canola) and synthetic CLA. The experiment involved eight treatments with a control (T1) consisting on the supply of commercial pelleted food *ad libitum* plus fresh alfalfa *ad libitum*; all the other treatments were with the same diet with the addition to the pelleted food of 8% of canola seed and 2% of fish oil (T2); 8% of canola seed (T3), 2% of fish oil (T4), 8% of canola, 2% of fish oil and synthetic CLA (T5), 6% of flax seed (T6), 2% of high oleic sunflower oil (T7) and 6% of chia seed (T8). 160 V line rabbits with an average weight of 1145g were randomly distributed to the different treatments. Each treatment included five cages containing four individuals each (2 males and 2 females). The rabbits were weekly weighted until they reach 2500g when they were slaughtered. *Longissimus lumborum* samples of five animals of each treatment were analyzed for intramuscular fat content and composition. Some rates were calculated to assess the fat nutritional value: PUFA/SFA, SFA/(MUFA+PUFA), n6/n3 and the atherogenicity and thrombogenicity indices. The addition of the evaluated ingredients showed modifications on the intramuscular fat composition when compared to the control (T1). The chance of improving the nutritional quality of rabbit meat through diet modifications with the goal of enriching meat contents in n-3 fatty acids, oleic acid and/or conjugated linoleic acid was confirmed. Fat composition differences between treatments also established effects on the indices

used to estimate the nutritional value. The sensory evaluation showed a negative influence of the fish oil on meat smell, taste and overall liking.

Key words: rabbit, diet, CLA, lipid profile, intramuscular fat, atherogenicity index, thrombogenicity index.

Introduction

The use of fresh forage produced on the farm as a partial substitute for commercial pelleted food is a traditional feeding strategy used by breeders to reduce feed costs in meat rabbit production in Uruguay (Amoza et al, 2008). Previous research in INIA Uruguay showed that the supply of fresh alfalfa *ad libitum* along with commercial pelleted food also *ad libitum* determined a significant reduction in commercial pelleted food intake and decreased feed cost of growing rabbits. These studies also allowed verifying that including alfalfa in the diet determined an increase in the content of linolenic acid and an improved ratio n6/n3 at intramuscular and dissectible fat (Capra et al., 2013).

The effect of diet composition on the rabbit meat lipid profile and nutrients content has been confirmed by numerous authors and has led to the search for dietary modifications that contribute to improve the meat nutritive value. Numerous research papers have focused their objective in the management of diet composition to increase the content of rabbit meat in omega-3 polyunsaturated fatty acid, improve n-6/n-3 ratio and enrich the content of bioactive compounds such as EPA, DHA, CLA, vitamin E and selenium (Lo Fiego et al., 2005; Gigaud and Le Cren, 2006; Marounek et al., 2007; Maertens et al., 2008; Tres et al., 2008; Peiretti and Meineri, 2008; Zsédely et al., 2008; Kowalska, 2008; Bielanksi and Kowalska, 2008). Rabbit meat can be a good way to provide consumers healthy compounds, with promising potential as a functional food (Dalle Zotte and Széndro, 2010; Hernández, 2012).

The present work was aimed at evaluating the effect of the addition of synthetic CLA, different oilseeds (chia, flax, canola), fish oil, high oleic sunflower oil and some combinations of these ingredients in the composition of the intramuscular fat of rabbits fed with a diet of commercial pelleted food and fresh alfalfa offered *ad libitum*.

Materials and methods

160 V line rabbits with an average weight of 1145g and 45 days old were randomly distributed to eight different dietary treatments, after a week of habituation to a combined diet of commercial pelleted food *ad libitum* plus fresh alfalfa *ad libitum*. Each treatment included five cages containing four individuals each (2 males and 2 females). Galvanized wire cages 0.86 x 0.40 x 0.33 m (length x width x height) were used for the growing-fattening period up to a slaughter weight of 2500g. The applied treatments had the following details:

T1. Commercial pelleted food *ad libitum* + fresh alfalfa *ad libitum*
T2. Commercial pelleted food *ad lib* with 8% canola seed + 2% fish oil + fresh alfalfa *ad lib*
T3. Commercial pelleted food *ad lib* with 8% canola seed + fresh alfalfa *ad lib*
T4. Commercial pelleted food *ad lib* with 2% fish oil + fresh alfalfa *ad lib*
T5. Commercial pelleted food *ad lib* with 8% canola seed + 2% fish oil + synthetic CLA 0,5% + fresh alfalfa *ad lib*
T6. Commercial pelleted food *ad lib* with 6% flax seed + fresh alfalfa *ad lib*
T7. Commercial pelleted food *ad lib* with 2% high oleic sunflower oil + fresh alfalfa *ad lib*
T8. Commercial pelleted food *ad lib* with 6% chia seed + fresh alfalfa *ad lib*

Ingredients of the commercial pelleted food used in this experiment are shown on Table 1.

Table 1.- Ingredients of the commercial pelleted food

Ingredients	kg
Alfalfa hay	360
Wheat bran	120
Corn	111.7
Sunflower meal	100
Weath middlings	80
Soybean meal	70
Wheat flour	70
Oats	60
Dicalcium phosphate	13.1
Calcium carbonate	5.6
Salt	5.4
Vitamin-mineral premix VM-602	2
Calcium proponiate	1
Zinc bacitracin	0.5
DL-methionine 99%	0.4
L-Lysine 95%	0.3
	1000

Chemical composition of the commercial pelleted food on a dry matter basis was 21.3% Crude Protein, 27% Acid Detergent Fiber, 38% Neutral Detergent Fiber, 3,6% Ether Extract, 1,9% Calcium and 1,1% Phosphorus.

The oilseeds (canola, chia, flax) were ground and mixed with commercial pelleted food. Oils (high oleic sunflower, fish) were mixed with pellets. Synthetic CLA used in the treatment T7 was Tonalin TG 80® from BASF, which contains a 50:50 mixture of two isomers (C18:2, c9, t11 and C18: 2, t10, c12). The supply of synthetic CLA in T5 treatment began on the fourth week of testing, in a dose of 5g per Kg of commercial pelleted food.

Fresh alfalfa was cut daily and the quantity supplied to each cage was weighed. The ration provided was weighed at the beginning of the week. The weighed quantities offered throughout the week and the rejection at the end of the week, were recorded to know the consumed quantity by difference. Actual consumption of alfalfa was not determined, registering only the alfalfa volume offered. Table 2 shows fresh alfalfa and feeds lipid profile.

Table 2. Feeds lipid profile (%)

	Alfalfa	T1	T2	T3	T4	T5	T6	T7	T8
Fatty acid	%	%	%	%	%	%	%	%	%
14:0	0.7	-	1.7	0.2	2.1	2.2	-	0.1	0.2
15:0	0.2	-	0.2	-	0.2	0.2	-	-	
15:1	0.3	-	0.2	-	0.2	0.3	-	-	
16:0	16.8	12.1	12.5	12.9	15.3	14.5	11.6	7.7	14.4
16:1	0.2	-	2.1	0.1	2.4	2.8	-	0.1	0.2
17:0	0.2	-	0.1	0.1	0.1	-	-		0.1
17:1			0.1	-	0.1	-	-		
18:0	2.5	2.6	2.3	2.4	2.6	2.6	2.3	2.6	2.8
18:1 n9c	1.8	26.8	38.3	27.2	27.1	27.4	23.9	61.7	27.3
18:2n6c	16.3	47.0	24.7	47.8	30.7	22.7	40.3	23.6	47.3
20:0	0.5	0.4	0.4	0.4	0.4	0.3	0.4	0.3	0.5
20:1		0.3	1.7	0.4	1.9	1.8	-	0.2	0.3
21:0									0.4
18:3n3	52.0	5.9	5.2	6.3	4.0	4.8	6.5	1.6	5.6
CLA						4.7	-	-	-
20:2			0.9	-	-	-	-	-	
22:0	0.6	0.3	0.2	-	0.2	-	-	0.5	0.3
20:3									0.6
20:4	0.8		-	-	-	-	-	-	
20.5n3		-	1.7	-	2.2	2.2	-	-	
22:6n3			3.2		4.5	4.1	-	-	
Saturated	21.5	15.4	17.4	16.0	20.9	19.8	14.3	11.2	18.7
Monounsaturated	2.3	27.1	42.4	27.7	31.7	32.3	23.9	62	27.8
Polyunsaturated	70.3	52.9	35.7	54.1	41.4	33.8	46.8	25.2	52.9
Total identified	94.1	95.4	95.5	97.8	94.0	90.6	85.0	98.4	99.4

When the slaughter weight was reached (2500g) samples of *Longissimus lumborum* muscle of five animals randomly selected from each treatment were taken to analyze the intramuscular fat chemical composition. To determine the percentage of intramuscular fat the extraction method of Folch, Lees and Sloane (1957) amended (hexane/isopropanol 3:2) was used. Analysis of the fatty acid profile of fat was carried out by gas chromatography.

On the basis of the chemical composition of the intramuscular fat different indices were calculated, including polyunsaturated/saturated, saturated/unsaturated, n-6/n-3 ratios and the atherogenicity and thrombogenicity indices proposed by Ulbricht and Southgate (1991).

For statistical analysis of intramuscular fat content and lipid profile, the experimental unit was the individual. For data processing Infostat 2008 was used. Analysis of variance was performed using the Tukey test (α=0.05) for comparison of mean values.

A sensory analysis was performed evaluating taste, odor, texture and overall liking of meat samples of four treatments (T1, T2, T3 and T4) by a consumer panel using a structured hedonic scale of nine points (1-dislike extremely, 5-neither like nor dislike, 9-like extremely). The evaluation was conducted in a standard ISO 8589:1988 room under white artificial light and controlled temperature ranging 22-24ºC, with a panel composed by 42 consumers where 43% were male. An analysis of variance for each attribute was performed and the minimum significant difference was calculated by Fisher's LSD ($p<0.05$) test using Infostat 2008 version.

Results and discussion

Table 3 shows the results of the intramuscular fat chemical composition analysis.

Table 3. Intramuscular fat content and fatty acid profile (%)

Treatment	T1	T2	T3	T4	T5	T6	T7	T8
% intramuscular fat	1,36 ± 0,24 a	1,46 ± 0,27 a	1,26 ± 0,17 a	1,60 ± 0,20 a	1,33 ± 0,10 a	1,62 ± 0,61 a	1,76 ± 0,25 a	1,34 ± 0,24 a
C14:0	1,95 ± 0,10 ab	1,92 ± 0,17 ab	1,55 ± 0,14a	2,19 ± 0,43b	1,78 ± 0,50 ab	2,02 ± 0,34 ab	1,67 ± 0,29 ab	1,75 ± 0,26 ab
C16:0	29,26 ± 1,63 b	26,68 ± 1,38 ab	28,04 ± 1,69ab	28,12 ± 1,43b	26,76 ± 1,86 ab	27,86 ± 0,69 ab	25,06 ± 1,50 a	28,21 ± 1,38 b
C18:0	5,29 ± 0,47*	5,82 ± 1,13a	5,17 ± 0,52a	5,37 ± 0,53a	6,13 ± 0,60 a	5,62 ± 0,78 a	5,79 ± 0,66 a	5,31 ± 0,46 a
C18:1 n9	27,25 ± 0,47 a	28,83 ± 3,00a	28,64 ± 2,24 a	26,26 ± 0,96 a	26,88 ± 2,78 a	26,29 ± 1,20 a	33,10 ± 0,95 b	25,21 ± 1,34 a
C18:2 n6	23,15±1,10 ab	20,23±1,39a	23,50±0,92 ab	21,49±2,36 ab	20,14±2,61 a	23,63±1,44 b	23,38±0,87 ab	22,37±1,49 ab
C18:3 n3	4,17 ±1,32 a	3,95 ±3,00 a	4,32 ±2,24 a	4,64 ±0,96 a	4,43±2,78 a	6,55±1,20 ab	3,89 ±0,95 a	7,78 ±0,85 b
C20:5 n3	0,17 ± 0,05 ab	0,58 ± 0,11 c	0,22 ± 0,07ab	0,58 ± 0,08 c	0,64 ± 0,03 c	0,21 ± 0,13 ab	0,14 ± 0,11 a	0,34 ± 0,11 b
C22:5 n3	0,78 ± 0,19 ab	1,05 ± 0,24 b	0,81 ± 0,12ab	0,85 ± 0,14 ab	1,18 ± 0,82b	0,70 ± 0,40 ab	0,51 ± 0,14a	1,15 ± 0,31 b
C22:6 n3	0,12±0,04 a	3,37 ±1,30 b	0,13 ±0,03 a	2,67 ±0,79 b	3,84 ±1,44b	0,13 ±0,09 a	0,10 ±0,03 a	0,17 ±0,04*
Σ C20:5+C22:5+C22:6 n3	1,06 ± 0,20 a	5,00 ± 1,27 b	1,16 ± 0,14 a	4,10 ± 0,76 b	5,66 ± 1,11 b	1,04 ± ,52 a	0,75 ± 0,13 a	1,66 ± 0,35 a
CLA 1*					0.49 ± 0,09			
CLA 2*					0,28 ± 0,05			
Σ n6	27,2 3± 1,43 ab	24,17 ± 1,69a	27,8 2± 0,46b	24,61 ± 2,59ab	24,40 ± 1,15a	26,84 ± 1,09ab	26,64 ± 0,78 ab	26,73 ± 1,50 ab
Σ n3	5,40 ± 0,64*	9,01 ± 1,49b	5,61 ± 0,89a	8,79 ± 1,74 b	10,19 ± 1,53b	7,75 ± 0,82ab	4,84 ± 0,81a	9,35 ± 1,33 b
SFA	37,43 ± 1,20 b	35,34 ± 1,80 ab	35,92 ± 1,26 ab	36,78 ± 1,04 ab	35,77 ± 1,43 ab	36,53 ± 0,39 ab	31,47 ± 0,81 a	36,19 ± 1,30 ab
MUFA	29.94 ± 1,18 a	31.49 ± 1,77 ab	30.64 ± 1,26 ab	29.83 ± 1,03 a	28.91 ± 1,43 a	28.89 ± 0,38 a	34.94 ± 0,81 b	27.40 ± 1,27 a
PUFA	32,62 ± 1,16 ab	33,18 ± 2,50 ab	33,43 ± 1,61 ab	33,40 ± 1,82 ab	34,56 ±1,71 ab	34,59 ± 1,00 ab	31,47 ± 0,80 a	36,42 ± 0,94 b

*CLA 1 corresponds to isomer C18:2, c9, t11 and CLA 2 to isomer C18:2, t10, c12
Different letters on the same column indicate significant differences P<0.05

T1. Commercial pelleted food *ad libitum* (CPF) + fresh alfalfa *ad libitum*;
T2. CPF with 8% canola seed + 2% fish oil + fresh alfalfa *ad libitum*;
T3. CPF with 8% canola seed + fresh alfalfa *ad libitum*;
T4. CPF with 2% fish oil + fresh alfalfa *ad libitum*;
T5. CPF with 8% canola seed + 2% fish oil + synthetic CLA 5g/Kg of pelleted food + fresh alfalfa *ad libitum*;
T6. CPF with 6% flax seed + fresh alfalfa *ad libitum*
T7. CPF with 2% high oleic sunflower oil + fresh alfalfa *ad libitum*;
T8. CPF with 6% chia seed + fresh alfalfa *ad libitum*

The addition of synthetic CLA in T5 is reflected as it is the only treatment with measurable amounts of the two isomers of linoleic acid. CLA levels in the intramuscular fat are lower than those obtained by Lo Fiego et al. (2005) using levels of incorporation of the synthetic product of 0,25 and 0,5%, that resulted in an increase in SFA content in meat. Marounek et al. (2007) also reported an increase in meat SFA at the expense of monounsaturated fatty acids (MUFA) with dose and timing of administration of CLA equivalent to those used in this experiment. Unlike the results obtained by the cited authors, in this study was not observed a significant increase in the content of SFA as an effect of the inclusion of CLA.

The inclusion of fish oil in the diet of rabbits determined an increase of relevant magnitude in the content of EPA, DPA and DHA, whereas the addition of high oleic sunflower oil determines a statistically significant increase in the content of C18:1 n9 and in total MUFA. Including chia seed determined a significant increase in the content of α-linolenic acid (ALA) and in total n-3 with respect to the control, although the values fall significantly below those reported by Peiretti and Meineri (2008) with inclusion levels of 10 and 15%. The addition of flax seed and canola seed determined smaller magnitude changes compared with control.

Dal Bosco et al. (2014) suggest that the increase in the intake of α-linolenic acid could increase the activity of the desaturase/elongase, causing an increase in fatty acid content of long-chain n3 fatty acids (EPA and DHA), but state that is not well known the level of efficiency in rabbit to elongate and desaturate ALA. Meanwhile Zsédely et al. (2008) achieved significant increases in the content of intramuscular fat ALA by including sunflower and flax oils, which also resulted in significant increases in the content of long chain n3. In this study the significant increase in the content of ALA achieved with treatment with chia (T8) compared with the control, is not accompanied by a statistically significant increase in the content of any of the n3 long-chain (EPA, DPA, DHA) neither in their sum.

From the results of this research it is clear that intramuscular fat significant levels of DHA in rabbits are only attained supplementing with fish oil (T2, T4 and T5), while diets rich in ALA do not determined nutritionally significant increases in long chain n3 fatty acids.

Different ratios between the proportions of fatty acids habitually used to characterize the nutritional value of the foods are presented in Table 4.

Table 4. Indicators of intramuscular fat nutritive value

Treatment	PUFA/SFA	n6/n3	SFA/(MUFA+PUFA)	AI	TI
T1	0,87±0,06 a	5,04 ±1,01bc	0,60 ±0,03 b	0,59±0,03 b	0,73 ±0,04 b
T2	0,94 ±0,05 a	2,68 ±0,52 a	0,55 ±0,04 ab	0,53 ±0,04 ab	0,60 ±0,05 a
T3	0,93 ±0,03 a	4,96 ±0,61 bc	0,56 ±0,03 ab	0,54 ±0,03 ab	0,64 ±0,04 ab
T4	0,91 ±0,10 a	2,80 ±1,01 a	0,58 ±0,03 ab	0,58 ±0,04 b	0,64 ±0,05 ab
T5	0,97 ±0,04 a	2,39 ±0,71 a	0,57±0,04 ab	0,54 ±0,05 ab	0,58 ±0,04 a
T6	0,94 ±0,04a	3,50 ±1,72 ab	0,57 ±0,01 ab	0,57 ±0,02 ab	0,65 ±0,04 ab
T7	0,93 ± 0,04 a	5,50 ±0,71c	0,51 ±0,02 a	0,48 ±0,02 a	0,64 ±0,03 ab
T8	1,00 ±0,07 a	2,90 ±1,73 a	0,57 ±0,03 ab	0,55 ±0,02 ab	0,60 ±0,06 a

Different letters on the same column indicate significant differences P<0,05
Atherogenicity Index: AI= [C12:0 + (4*C14:0) + C16:0] / [(ΣPUFA) + (ΣMUFA)]
Thrombogenicity Index: TI= [C14:0 + C16:0 + C18:0] / [(0.5*ΣMUFA) + (0.5* Σ n-6) + (3*Σn-3)+ (n-3/n-6)]

By analyzing the results from the point of view of the nutritive value, all treatments show low intramuscular fat content, with values less than 1,8g/100 g meat. Lipid profiles are favorable in all treatments in relation to SFA, MUFA and PUFA consumption recommendations made by FINUT-FAO, 2012. The PUFA/SFA ratios were above the 0.45 recommended by the World Health Organization, with no significant differences between treatments. The n6/n3 ratio values were less than or very close to the recommended value of 5, but in this case treatment with fish oil (T2, T4 and T5) and chia seeds (T8) showed significant differences with the control (T1). For the saturated/unsaturated ratio, significant differences were only verified between the control (T1) and treatment with high oleic sunflower oil (T7) due to the significantly higher content of monounsaturated fatty acids of this oil.

Dal Bosco et al. (2014) state that rabbit meat lipid profile has remarkable nutritional attributes that would allow it to be considered as a healthy meat. They emphasize that the addition of fresh alfalfa to the commercial pelleted food significantly modifies the content of ALA, EPA and DHA and reduces n6/n3 ratio and the atherogenicity and thrombogenicity indices. In this paper, atherogenicity and thrombogenicity indices obtained on all treatments are below those determined by Dal Bosco et al. (2014). In contrast, all indices determined in this work are above the values obtained by Peiretti and Meineri (2008) in diets with 10 and 15% of chia seed.

The way of modifying the commercial pelleted food by mixing the pellets with oil or ground seeds was effective, which would allow rabbit breeders to make dietary changes without searching for already modified commercial pelleted food. The adoption of these dietary modifications will be conditioned by economic factors and by the possible consumer demand for rabbit meat enriched with bioactive compounds.

Besides the economic and health issues of rabbit meat, consumer demand will be driven by meat sensory quality. The results of sensory evaluation are presented in Table 5.

Table 5. Sensory evaluation results

Treatment	Taste	Odor	Texture	Overall liking
T3	6.8 a	6.4 a	6.6 a	6.8 a
T1	6.7 a	6.0 ab	6.8 a	6.6 a
T4	5.9 b	5.5 b	6.6 a	6.1 ab
T2	5.7 b	5.4 b	6.5 a	5.8 b

Different letters on the same column indicate significant differences $P<0.05$

The samples corresponding to treatments without fish oil (T1 and T3) showed significantly better scores than those including fish oil (T2 and T4). Scores obtained for T3 treatment including canola tended to be better evaluated in taste, odor and overall liking than the control T1, although the differences were not significant for any of the parameters evaluated. All attributes were judged with positive notes, above the 5 corresponding to an assessment of indifference, although panelists mostly had little or no previous experience in the consumption of rabbit meat.

Conclusions

The results obtained confirm the ability to positively modify the lipid profile of rabbit meat intramuscular fat by dietary management. An improvement was observed in the content of n3 long chain fatty acids by adding fish oil on the diet. The results suggest that to drive EPA and DHA through the rabbit intramuscular fat with the purpose of using rabbit meat as an optional substitute of fish, is necessary to study in-depth technological alternatives that allow masking the undesirable effects of fish oil on the sensory properties of rabbit meat. The intramuscular fat ALA increase at treatments that added chia and flax seeds did not determine a statistically significant increase in the content of EPA and DHA compared to the control. The addition of high oleic sunflower oil increased oleic acid C18:1 n9 which in turn significantly improves the atherogenicity index and the SFA/(MUFA+PUFA) ratio.. The inclusion of synthetic CLA can enhance intramuscular fat with this bioactive compound.

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