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DIET DIGESTIBILITY IN GROWING RABBITS: EFFECT OF ORIGIN AND OXIDATION LEVEL OF DIETARY FAT AND VITAMIN E SUPPLEMENTATION

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Abstract: The effects of the dietary inclusion of fats with different origin (lard or vegetal oil), fatty acid profile (linseed or sunflower), oxidation level (fresh, peroxidised: 11 d at 55ºC or oxidised: 31 h at 140ºC) and vitamin E supplementation (0 or 100 ppm) on the rabbit diet apparent digestibility were studied. Digestibility coefficients of dry matter, organic matter, crude protein, ether extract and gross energy were determined in eight diets using 58 rabbits aged 49 d. Contrast analysis between groups of diets showed that lard, characterised by a greater saturated fatty acid content, compared with vegetal oils, rich in unsaturated fatty acid, reduced the apparent digestibility of ether extract (62.3 *vs*. 68.4%; *P*=0.0329). However, there were no significant differences in the nutrient digestibility when linseed or sunflower oils (rich in ω-3 or in ω-6 polyunsaturated fatty acids, respectively) were compared. The oxidation degree of the sunflower oil and the supplementation with 100 ppm of vitamin E to the diets did not modify the apparent digestibility values of any dietary fraction.

Key Words: rabbit, fat, ω-3/ω-6 fatty acids, oxidation level, tocopherol, digestibility.

INTRODUCTION

The inclusion of fats in diets for rabbits has effects upon the productivity and development of the animal that determine the interest in their use. Several studies (reviewed by Fernández-Carmona *et al*., 2000) have demonstrated that the tolerance of these animals to dietary fats is high if the diets are of good quality, since they improve feed palatability and are well digested by the animal. The authors recognise some important properties for the use of fat in diets: increased dietary energy, highly effective energy metabolisation and provision of essential fatty acids. It allows the energy content of the diet to be increased without reducing its level of fibre (Fernández-Carmona *et al*., 2000), a highly important nutrient for the correct transit and digestive functioning in rabbit, resulting generally in a reduction of production costs.

The effect of fat type on the digestive utilisation of diets has been widely studied in swine and chickens (Li *et al*., 1990; Preston *et al*., 2001) but there is less information in rabbit. Almost all authors find that dietary digestibility increases when fat is included in the diet (Xiccato, 1998; Bhatt and Swain, 2003; Cesari *et al*., 2009) and that a greater degree of non saturation of fatty acids (FA) in fat enhances the apparent digestibility of this fraction (Maertens *et al*., 1986; Santomá *et al*., 1987). This may be due to the better emulsion and absorption of unsaturated fats in the digestive tract (Hakanansson, 1974; Dolz, 1996). However, Fernández *et al*. (1994) suggested that the effect may be more complex and that the unsaturated/saturated FA ratio is not the most suitable indicator to measure digestibility, especially if the fat source

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contains lipids bonded to the cellular walls (as occurs with most conventional raw materials). However, no studies have been done in rabbit to compare digestibility of polyunsaturated fat varying in fatty acid profile, rich in ω-3 or ω-6 polyunsaturated fatty acids (PUFA). Moreover, high dietary fat inclusion levels could also interfere negatively with digestive efficiency and the activity of caecal microflora, with the result that the cited increase would not be linear (Fernández-Carmona *et al*., 1996; Pascual *et al*., 1998; Falcao-e-Cunha *et al*., 2004).

On the other hand, almost all the works that have evaluated fats in monogastric feeding assume that they are not deteriorated and are of good quality, while it is admitted that a high polymer content supposes a lower nutritional value of the fat, or that a higher peroxide content entails a greater consumption of natural antioxidants, such as vitamins E and C (Dolz, 1996). However, Choque-López (2008) studied the effect of fat oxidation on the digestive utilisation of diet in chickens, without finding differences in the faecal digestibility of dry matter (DM) or ether extract (EE). Similar studies are needed in rabbit, since there are no available data, including the possible interference of the addition of vitamin E to the diet, because of its antioxidant role stabilising the unsaturated FA (Pryor, 2000).

The aim of the present work was to study the effect of the inclusion of fats varying in fatty acid profiles or in oxidation levels, with or without vitamin E supplementation, on the dietary digestibility in fattening rabbits.

MATERIAL AND METHODS

Diets

To assess the digestibility of the diet according to the fat type, degree of fat oxidation and addition of vitamin E, eight experimental diets were formulated following the nutritional recommendations for fattening rabbits issued by de Blas and Mateos (1998). The ingredients of the diets and the different experimental treatments studied are shown in Table 1. All diets had the same basal mixture of ingredients and only varied in the type of fat included: lard (diet A), linseed oil (rich in ω-3 FA: linolenic acid; diet L), sunflower oil (rich in ω-6 FA: linoleic acid; diet S), peroxidised sunflower oil (diet SP), oxidised sunflower oil (diet SO), and S, SP and SO diets supplemented with 100 ppm vitamin E (diets SE, SPE and SOE). All diets included a mineral-vitamin premix that supplied 20 ppm vitamin E.

Table 1: Ingredients of diet and experimental treatments.

Ingredients	g/kg DM	Ingredients	g/kg DM
Wheat bran	150	L-lysine HCL	3
Beet pulp	280	L-threonine	1
Alfalfa hay	250	Calcium carbonate	2
Sunflower meal	200	Dicalcium phosphate	12
Soybean meal	60	Sodium chloride	5
Fat (according to treatment) [1]	30	Robenidine[2]	1
DL-methionine	1	Vitamin/mineral mixture[3]	5

[1] Treatments, A: Animal fat (lard), L: Linseed oil, S: Sunflower oil, SE: S and 100 ppm Vitamin E, SP: Peroxidised sunflower oil, SPE: SP and 100 ppm Vitamin E, SO: Oxidised sunflower oil, SOE: SO and 100 ppm Vitamin E

[2]Contain: 66 mg/kg

[3]Per kg of feed: Vitamin A: 8,375 IU; Vitamin D3: 750 IU; Vitamin E: 20 mg; Vitamin K3: 1 mg; Vitamin B1: 1 mg; Vitamin B2: 2 mg; Vitamin B6: 1 mg; Nicotinic acid: 20 mg; Choline chloride: 250 mg; Mg: 290 mg; Mn: 20 mg; Zn: 60 mg; I: 1.25 mg; Fe: 26 mg; Cu: 10 mg; Co: 0.7; Butyl hydroxylanysole+ethoxiquin: 4 mg.

Table 2: Effect of thermal treatment on oxidation level and fatty acid composition of sunflower oil (Tres *et al.,* 2009).

	Fresh	Peroxidised	Oxidised
Thermal treatment	No	55ºC, 11 d	140ºC, 31 h
Evaluation of oil oxidation[1]			
Peroxide value	10.4	83.0	9.80
p-anisidine value	2.80	2.70	124.5
Polymer content	0.09	0.25	9.90
Fatty acids profile[2], g/kg oil			
SFA	110	108	106
MUFA	188	189	184
PUFA	632	622	582
Total *trans* fatty acids	1.06	1.08	1.87

[1]Peroxide value, mEq O_2/kg oil; Polymer content, % w/w.
[2]SFA (saturated fatty acids), sum of C14:0, C16:0, C17:0, C18:0, C20:0, C22:0 and C24:0; MUFA (monounsaturated fatty acids), sum of C16:1ω-9, C18:1ω-9, C20:1ω-9, C16:1ω-7 and C18:1ω-7; PUFA (polyunsaturated fatty acids), sum of C18:2ω-6, C20:2ω-6 and C18:3ω-3; Total *trans* fatty acids, sum of *c*9, *t*12-18:2, *c*9, *t*11-CLA, *t*10 and *c*12-CLA and mixture of di-*trans* conjugated linoleic acid.

Three aliquots of non-refined sunflower oil were used for thermal treatments. The first aliquot did not undergo any thermal treatment and was used as fresh oil. Peroxidised oil was prepared by heating the second aliquot at 55 ºC for 11 d with agitation, to obtain an oil with a high content of primary oxidation compounds (peroxides), whereas the oxidised oil was prepared by heating the third aliquot in a direct fryer at 140 ºC for 31 h, obtaining an oil rich in secondary oxidation compounds (aldehydes, alcohol, ketones, acids and hydrocarbons). Immediately after the thermal treatments, butyl hydroxytoluene was added to the oils at 100 ppm to avoid further oxidation. Fatty acid composition and several oxidation parameters were measured in order to characterise the oils (Table 2, showing data from the same samples used in this study analysed by Tres *et al*, 2009).

Animals and experimental desing

The digestibility assay was carried out following the methodology proposed by EGRAN (Perez *et al*., 1995) and 58 rabbits were used (7-8 animals per diet). The assay began at weaning (28 d of life) with eighty animals, lodged individually in digestibility cages and fed one of the eight diets under study until the end of the experimental period.

At 42 d of age, the animals with an initial weight of 1258 ± 143 g were monitored for correct consumption and growth for 7 d, after which feed intake was measured and faeces were collected for 4 d, from 49 to 53 d of age, and stored at −18ºC until drying and chemical analyses. Animals with abnormal consumption or growth figures and symptoms of illness from 42 to 53 d of life were removed from the experiment.

Chemical Analyses

Feed and faeces were analysed using the AOAC methods (2000) and following the recommendations of EGRAN (2001). DM was determined following AOAC official method 934.01, crude protein (CP) by using a Kjeltec 2300 analyser (Foss, Sweden) and following AOAC official method 976.05, EE and ash contents following the protocols described by the AOAC methods 920.39 and 942.05, respectively, and

gross energy (GE) content was determined by combustion in adiabatic calorimetric pump, according to the recommendations of EGRAN (2001).

Crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) of diets were determined using filters bags and a Fiber Analyzer A220 (ANKOM, USA) and following AOAC official methods, 978.10 for CF and 973.18 for ADF and ADL (AOAC, 2000) and Mertens (2002) for NDF. The fatty acids content of diets A, L, S, SP and SO was determined extracting the fat according to Folch *et al*. (1957) and derivatising the FA to obtain their methyl esters according Morrison and Smith (1964). Methyl esters were analysed in a Focus Gas Chromatograph (Thermo, Milan, Italy), equipped with a split/splitless injector and a flame ionisation detector. Separation was performed in an SPTM 2560 capillary column (Supelco, PA, USA) (100 m×0.25 mm×0.2 µm film) with a helium flow of 1.1 mL/min, according to the following temperature gradient: 140ºC initial temperature for 5 min, followed by a linear gradient of 4ºC/min until reaching 240ºC, temperature which was maintained for 30 min. The detector and injector were kept at 260ºC. The chemical composition and the fatty acid profiles of experimental diets are shown in Table 3.

Statisitical Analyses

The daily DM intake and apparent digestibility coefficients obtained during the experimental period were statistically analysed by analysis of variance following the GLM procedure (SAS, 2002), considering the diet as main factor. In addition, seven non-orthogonal contrasts were performed: i) added lard *vs.* vegetal oils [A−1/7(L+S+SE+SP+SPE+SO+SOE)]; ii) linseed oil *vs.* sunflower oil [L−S]; iii) vitamin E addition

Table 3: Chemical composition of diets (% DM) and fatty acids composition of dietary fat (%).

	Diets [1]							
	A	L	S	SE	SP	SPE	SO	SOE
Dry matter (DM, %)	92.6	92.7	93.2	91.2	93.5	92.4	92.5	92.8
Ash	10.2	10.9	9.9	10.9	9.9	9.6	10.0	10.2
Ether extract	5.09	5.14	4.80	4.17	5.12	3.96	5.20	4.5
Crude protein	18.9	18.7	18.2	17.9	18.5	17.9	18.6	18.6
Crude fibre	19.6	19.7	18.3	20.1	19.1	19.9	18.5	20.6
Neutral detergent fibre	39.6	40.1	40.1	43.7	39.4	42.9	39.8	42.4
Acid detergent fibre	23.1	22.7	23	23.9	22.6	26.4	23.6	26.1
Acid detergent lignin	6.1	5.3	6.8	5.5	4.7	6.7	6.6	6.8
Gross Energy (MJ/kg DM)	18.05	18.40	18.36	18.36	17.88	18.54	17.93	18.86
C14:0	1.88	0.28	0.30		0.31		0.16	
C16:0	25.01	11.07	11.60		11.86		11.96	
C16:1	1.25	0.12	0.16		0.13		0.12	
C18:0	11.44	2.85	3.39		3.42		3.49	
C18:1 (ω-9)	29.58	17.20	18.44		18.39		18.69	
C18:1 (ω-7)	0.17	0.34	0.31		0.31		0.37	
C18:2 (ω-6)	26.21	29.80	62.30		61.94		61.68	
C18:3 (ω-3)	4.46	38.34	3.50		3.64		3.53	

[1] Including 30 g/kg DM of A: lard, L: linseed oil, S: sunflower oil, SE: S+100 ppm vitamin E, SP: peroxidised sunflower oil, SPE: SP+ 100 ppm vitamin E, SO: oxidised sunflower oil, or SOE : SO+100 ppm vitamin E.

(E) *versus* no addition (0E) [1/4(SE+SPE+SOE)−1/4(S+SP+SO)]; iv) fresh sunflower oil (F) *versus* heated sunflower oils (H) [1/2(S+SE)−1/4(SP+SPE+SO+SOE)]; v) peroxidised sunflower oil (P) *versus* oxidised sunflower oil (O) [1/2(SP+SPE)−1/2(SO+SOE)]; vi) interaction between vitamin E addition and oil alteration [1/2(S+1/2(SPE+SOE))−1/2(SE+1/2(SP+SO)]; and vii) interaction between vitamin E addition and oil oxidation level [1/2(SP+SOE)−1/2(SPE+SO)]. Results were presented as least squared means.

RESULTS AND DISCUSSION

The apparent digestibility coefficients as well as the feed intake during the digestibility period are shown in Table 4.

Effect of Fat Origen

No differences were detected in digestibility coefficients of DM, organic matter (OM), GE or CP depending on the type of added fat (lard *vs*. vegetal oil). However, the apparent digestibility of EE was 9 % lower ($P<0.05$) for lard, which has more saturated fatty acids (C16:0 and C18:0, Table 3), compared with vegetal oils, richer in unsaturated fatty acids (C18:2 and C18:3, Table 3). Among vegetal oils, no differences were observed in the digestibility of considered nutrients depending on the type of polyunsaturated fatty acids (L *vs*. S), i.e. between linseed oil rich in ω-3 and sunflower oil rich in ω-6.

The lack of effect of type of fat on the digestibility of DM, OM, GE and CP in diets including only 3% of added fat is in agreement with Fernández *et al*. (1994). Other authors have recorded differences in the EE digestibility in growing rabbits similar to that found in the current work, when lard was compared with soybean oil (Maertens *et al*., 1986), or tallow was compared with soybean oil (Fernández *et al*., 1994). In the current study, the differences between digestibility coefficients of lard and vegetal oils could be estimated at around −10 points of percentage, near to −12 points recorded for the mentioned comparisons.

Table 4: Intake (g dry matter/d) and apparent digestibility coefficients (%) of diets and contrast according to type of fat.

	Diets[1]									Contrast[3]
	A	L	S	SE	SP	SPE	SO	SOE	RSD[2]	A *vs.* V
No. of animals	7	8	8	7	7	7	7	7		
Intake	129	122	122	135	130	124	133	123	13.5	0.6880
Dry matter	60.2	59.9	59.7	60.1	60.0	59.8	59.8	60.1	2.83	0.7786
Organic matter	61.0	60.5	61.0	60.9	60.8	61.1	60.7	61.1	2.83	0.8737
Crude protein	71.2	70.5	69.7	70.1	70.1	69.9	71.3	69.8	3.45	0.4229
Ether extract	62.3	67.1	67.5	69.9	67.9	70.3	68.7	69.4	7.67	0.0329
Gross energy	59.1	59.9	60.7	60.0	58.6	56.7	58.7	59.5	3.05	0.7433

[1] Including 30 g/kg dry matter of A: lard, L: linseed oil, S: sunflower oil, SE: S+100ppm vitamin E, SP: peroxidised sunflower oil, SPE: SP+100 ppm vitamin E, SO: oxidised sunflower oil, or SOE : SO+100ppm vitamin E.

[2] RSD: residual standard deviation.

[3]A *vs.* V: Lard compared with vegetable oils [diet A–1/7(diets S+L+SE+SP+SPE+SO+SOE)], Others not significant contrast; Linseed oil compared with sunflower oil [diets L–S], Vitamin E addition compared with not addition [diets (SE+SPE+SOE)–(S+SP+SO), Fresh sunflower oil compared with heated sunflower oils [diets (S+SE)–1/2(SP+SPE+SO+SOE)], Peroxidised sunflower oil compared with oxidized sunflower oil [diets (SP+SPE) – (SO+SOE)], Interaction between vitamin E addition and oil alteration [1/2(S+1/2(SPE+SOE))–1/2(SE+1/2(SP+SO))], Interaction between vitamin E addition and oxidation level [1/2(SP+SOE)–1/2(SPE+SO)].

The differences in EE digestibility, as occurs in other species, may be due to a negative correlation between the degree of saturation and fat digestibility, probably because the unsaturated fats are more easily emulsified and, therefore, better digested and absorbed in the intestine (Hakanansson, 1974; Dolz, 1996). Nevertheless, it could be also partially due to interference of caecal microbiota hydrogenating not digested dietary PUFA that increase the faecal output of saturated fatty acids (Xicatto, 1998).

To the authors' knowledge, no studies have been previously performed comparing digestibility of linseed and sunflower oils, or in general ω-3 *versus* ω-6 rich oils. Our results seem to indicate that the position of double bonds in the fatty acid do not modify its digestive utilisation.

Effects of oxidation level and vitamin E supplementation

Thermal treatment of oil altered its oxidation and FA profile (Table 2). Heating the oil at 55ºC for 11 d led to an increase in the primary oxidation compounds, and the peroxide value rose from 10.4 to 83.0 mEq O2/kg oil, while secondary oxidation compounds remained at the level found in fresh oil. However, compared to fresh oil heating oil at 140ºC for 31 h increased *p*-anisidine value and the polymer and *trans*-FA contents, but peroxide value was reduced due to the instability of primary oxidation compounds at high temperature (Tres *et al*., 2009).

No significant effects of either oxidation level (fresh *vs*. heated, and peroxidised *vs*. oxidised) or vitamin E supplementation (E *vs*. 0E) were found on the apparent digestibility of different nutrients, probably because the fatty acid profile was very similar among all diets including sunflower oil, whether it was fresh, peroxidised or oxidised (Table 3). Fatty acid profiles of diets E (SE, SPE and SOE) were not determined because they were manufactured from the same unique mixture of raw material as diets 0E (S, SP and SO, respectively) and no change with respect to 0E diets can be expected, considering that they remained unvaried after heating oil for peroxidation or oxidation. Only an increase in the dietary level of *trans* fatty acids (C18:1(ω-9)*trans* and C18:2(ω-3)*trans*) in oxidised oil was detected compared to fresh and peroxidised oils, without having any effect on the digestive utilisation of the diet.

Different authors working on chicken nutrition have found controversial results. Lin *et al*. (1989) found that diets supplemented with oxidised fats had a greater risk of lipid deterioration and loss of nutritional value, and affected negatively feed intake and nutrient digestibility, whereas the use of antioxidants improved the results, although it should be noted that the levels of fat inclusion in chicken diets were higher than those used in rabbits (7% *vs*. 3%, respectively). Nevertheless, in another work in which oxidised oils recycled from frying restaurants were included in chicken diets at 6% (Choque-Lopez, 2008) no effect of the degree of oxidation of the dietary fat on the digestibility of the different nutrients was recorded. Marquez-Ruiz *et al*. (2008) reported high digestibility values in rats fed diets with thermo-oxidised oil. They explained it by a possible breakage of dimers and polymers, the major compounds formed under frying temperatures, in the stomach by depolymerisation reactions.

CONCLUSIONS

From the results obtained in this work, we conclude that the apparent digestibility of the ether extract of the diet is higher when vegetal oils instead of lard were included in the diets of fattening rabbits at levels of 3%. The higher content of ω-3 or ω-6 fatty acids, the oxidation level of the oil or the supplementation with 100 ppm of vitamin E do not significantly modify the apparent digestibility values of the diets.

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