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EFFECT OF OXIDATION STATE OF DIETARY SUNFLOWER OIL AND DIETARY ZINC AND α-TOCOPHERYL ACETATE SUPPLEMENTATION ON PERFORMANCE OF GROWING RABBITS

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ABSTRACT: Twelve diets were formulated using the same raw materials and including 3% of sunflower oil at 3 different oxidation levels [fresh (F), peroxidised (P; 55°C for 11 d; 83 meq O_2/kg oil) and highly-oxidised (O; 140°C for 31 h; ρ-anisidine value of 125)], with 2 levels of α-tocopherol supplementation (0 and 100 ppm), and 2 levels of Zn supplementation (0 and 200 ppm). A trial with 900 growing rabbits was carried out in order to study the effect of the oxidation and protection level of supplemented oil on the performance of animals from weaning (28 d) to 63 d of age. Another coetaneous trial was performed to study the effect of the oxidation level of sunflower oil (F, P and O) on caecal activity around weaning, using 120 suckling rabbits randomly re-allocated into 12 litters of 10 kits (4 litters per diet) from 17 to 44 d of age. Four rabbits per litter were slaughtered at 30 and 44 d (16 rabbits/treatment and age). Full gastro-intestinal tract and caecum were weighed and pH, dry matter (DM), ammonia nitrogen (NH_3) and volatile fatty acids concentration (VFA) values in caecal content were measured. No effect was observed either in the mortality rate, body weight gain, feed intake or conversion rate throughout the growing period when peroxidised or oxidised oils were included in the diet, being on average 32%, 45.1 g/d, 107.6 g DM/d and 2.44, respectively. Dietary supplementation with α-tocopherol and/or Zn had no effect on the mortality rate, feed intake and performance of rabbits during the fattening period. Daily weight gain just after weaning (28 to 30 d of age) was higher for kits receiving the diet supplemented with F and O diets than those with the P diet (55, 50 and 35 g/d, respectively; $P<0.05$), but no further effect on performance was observed. Young rabbits fed with the P diet showed lower DM percentage in caecum at 30 d of age (–9.5%; $P<0.05$) than those with F or O diets. Caecum of young rabbits fed with the O diet presented lower NH_3 content at 30 d of age that those given F diet (–38%; $P<0.05$) and higher total VFA and acetic acid concentration (+36 and +34 %, respectively; $P<0.05$). Therefore, and although many questions are still open, oxidised oils could be considered as a possible energy source for rabbit nutrition.

Key Words: rabbit growth, oil oxidation, Zn supplementation, α-tocopherol supplementation.

INTRODUCTION

The health benefits of polyunsaturated fatty acids (PUFA) in human nutrition have stimulated interest in increasing them in animal products by dietary supplementation. PUFA rich oils are nevertheless prone to lipid oxidation which can occur at low or high temperatures, as well as during long-term storage or frying. At low or moderate temperatures, hydroperoxides are the main products formed in the initial stages of oxidation, whereas at high temperatures (above

150°C), hydroperoxides are practically absent because they are immediately decomposed into secondary oxidation products (Hamilton and Kirstein, 2008). Several of these oxidation products have a range of detrimental biological effects (Staprans *et al.*, 2005), but the proportion of compounds coming from dietary oxidised lipids is unknown (Marquez-Ruiz *et al.*, 2008). Gastrointestinal tract (GIT) is exposed to dietary oxidised lipids and, although the possible modifications and effects occurring are often ignored (Kanner, 2007), they could affect animal growth and metabolism at high doses (Billek, 2000; Özpinar *et al.*, 2001).

A significant amount of recycled fried oils have traditionally been used in animal feeds (Ohlson, 1992). However, there is controversy on the possible effect of the dietary use of these products on animal performance and health, as well as public health. Although peroxides are probably poorly absorbed by the organism, they are quite unstable and can generate many free radicals which affect many cellular functions. On the contrary, aldehydes and other secondary products of fat oxidation are well absorbed and transported in the organism by lipoproteins and may be toxic to the liver, kidneys and spleen (Szarek *et al,* 2006). There is a large number of works evaluating the short- and long-term effects of the use of extremely overheated fats and oils, mainly in laboratory animal diets (e.g. in rats: Crampton *et al.*, 1953; Gabriel *et al.*, 1978; Eder and Kichgessner, 1999), but there is a lack of sufficient knowledge about the dietary use of recycled frying oils at commercial feed levels in livestock animals to assess their impact on animal performance, health and well-being.

The lipid oxidation degree of oil did not affect digestibility of nutrients in pigs (Derouchey *et al.*, 2000) or rabbits (Blas *et al.*, 2010; Casado *et al.,* 2010), and Zdunczyk *et al* (2000) described a lower fat digestibility only when high-oxidised fats (200 meq O_2/kg and ρ-anisidine value of 96) were included at 10% in diet for rats, but not affecting feed intake and body weight gain. Effects of oxidised fats on animal performance also seem to be related with their oxidation degree and inclusion level. Peroxide values below 100 meq O_2/kg of fats included at commercial levels did not depress feed intake or growth rate in broiler chickens (Cabel *et al.*, 1988; Pesti *et al.*, 2002), turkeys (Lea *et al.*, 1966), swine (Carpenter *et al.*, 1966) and rabbits (Blas *et al.*, 2010). However, nutrient digestibility and growth performance were sharply declined for high peroxide values in poultry (when 150 meq O_2/kg of fat; Engberg *et al.*, 1996) and weanling pigs (786 meq O_2/kg of fat; Yuan *et al.*, 2007).

In any case, such raw materials could negatively affect the oxidative status of the animal, and protection with antioxidants (as fat soluble vitamins or some minerals and quinolones) could be of assistance (Grau *et al.*, 2001; Tres *et al.,* 2010a). Cabel *et al.* (1988) recorded that adding 125 ppm of ethoxyquin alleviated the effects on performance that occurred when broilers were fed fat with 175 meq O_2/kg. Some minerals, such as Se and Zn, are also related to the antioxidant system because they are part of some antioxidant enzymes (glutathione peroxidise and superoxide dismutase, respectively). Zn supplements in chicken diets had no effect on Zn content and oxidative stability of meat, but increased Se level, which is interrelated with α-tocopherol in the antioxidant system, contributing to the decomposition of lipid hydroperoxides to less reactive hydroxyl or aldehydic compounds in the gastrointestinal tract (Bou *et al.*, 2004, Marquez-Ruiz *et al.*, 2008).

The present work was performed to contribute knowledge clarifying how the oxidation state of dietary sunflower oil included at commercial levels affects young rabbit performance in the usual fattening period conditions, evaluating 3 levels of oxidation (fresh, peroxidised and highly

oxidised), as well as the possible dietary protection against this oxidation with α-tocopherol and/or Zn supplementation.

MATERIALS AND METHODS

Experimental oils and diets

The peroxidised (P) and highly oxidised (O) oils were obtained from fresh (F) sunflower oil (peroxide value of 10 meq O_2/kg oil and ρ-anisidine value of 3) by 2 heat treatments (55°C for 11 d or 140°C for 31 h, respectively; see Casado *et al.,* 2010) which increased the content of primary oxidation compounds (P oil), as was reflected in a peroxide value of 83 meq O_2/ kg oil without variation of ρ-anisidine value, or induced the formation of secondary oxidation compounds and polymerisation reactions (O oil), reaching a ρ-anisidine value of 125, 10% of polymerised triacylglycerols and 1.9 g/kg oil of total *trans*-fatty acids. Immediately after the heat treatments, butyl-hydroxytoluene was added to the oils at 100 mg/kg for their stabilisation, avoiding further oxidation.

Twelve diets for growing rabbits were formulated following the recommendations of de Blas and Mateos (1998), using the same mixture of raw materials (15% wheat bran, 28% beet pulp, 25% alfalfa hay, 20% sunflower meal, 6% soybean meal and amino acids, vitamins and minerals; Table 1) and including 3% of the experimental oil. The 12 isocaloric, isofibrous and isoproteic dietary treatments were prepared in a factorial arrangement: 3 oil sources (F, P and O diets)×2 levels of α-tocopherol supplementation (E; 0 and 100 ppm)×2 levels of Zn supplementation (Z; 0 and 200 pm). Diets were pelleted and analysed twice during the trial.

Table 1: Treatment arrangement and average chemical composition of diets[1].

Diet	Oil	Zn (ppm)	Vit E (ppm)	Chemical composition	g/kg DM
F	Fresh	0	0	Dry matter (DM, g/kg)	914
FZ	Fresh	200	0	Ash	95
FE	Fresh	0	100	Ether Extract	51
FEZ	Fresh	200	100	Starch	57
P	Peroxidised	0	0	Crude Protein	195
PZ	Peroxidised	200	0	Crude Fibre	178
PE	Peroxidised	0	100	Neutral Detergent Fibre	454
PEZ	Peroxidised	200	100	Acid Detergent Fibre	231
O	Oxidised	0	0	Acid Detergent Lignin	66
OZ	Oxidised	200	0	Gross Energy (MJ/kg DM)	18.4
OE	Oxidised	0	100	Digestible protein[2]	137
OEZ	Oxidised	200	100	Digestible energy (MJ/kg DM)[2]	10.9

[1] Raw materials composition (%): 15 wheat bran, 28 beet pulp, 25 alfalfa hay, 20 sunflower meal, 6 soybean meal, 3 experimental oil, 0.1 DL-methionine, 0.3 L-lysine HCL, 0.1 L-threonine, 0.2 calcium carbonate, 1.2 dicalcium phosphate, 0.5 sodium chloride, 0.1 Cycostat® and 0.5 vitamin-mineral mixture (from Trouw Iberica S.A.,L510®).
[2] Values obtained from average apparent digestibility coefficients of gross energy and crude protein obtained for these same diets by Casado *et al.* (2010).

Feeds were analysed using AOAC methods (2000) and following EGRAN recommendations (2001). Dry matter (DM) was determined by the AOAC official method 934.01, crude protein (CP) by using a Kejltec 2300 analyser (Foss, Sweden) and AOAC official method 976.05, ether extract and ash contents following the protocols described by AOAC methods 920.39 and 942.05, respectively, while gross energy (GE) content was determined by combustion in adiabatic calorimetric pump, according to EGRAN recommendations (2001).

Starch content was determined according to Batey (1982), by a 2-step enzymatic procedure with solubilisation and hydrolysis to maltodextrins with thermo-stable α-amylase followed by complete hydrolysis with amyloglucosidase (both enzymes from Sigma-Aldrich, Steinheim, Germany), and the resulting glucose were measured by the hexokinase/glucose-6 phosphate dehydrogenase/NADP system (R-Biopharm, Darmstadt, Germany). 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 Analyser 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.

Experimental procedure

Housing, husbandry and slaughtering conditions followed the current recommendation on principles of ethical care and protection of animals used for experimental purposes in the European Union (2003) and all trials were subject to approval by the Animal Protocol Review Committee of the Polytechnic University of Valencia. The experiment was carried out following the recommendations for applied nutrition research in rabbits described by the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005).

A growing trial was performed with 900 weaned rabbits (75 animals per diet) aged 28 d that were randomly housed in individual cages (26×50×31 mm high). All the rabbits had free access to one of the 12 experimental diets from 28 to 63 d of age and live weight (LW) at 28 and 63 d, daily weight gain (DWG), daily feed intake (DFI) and feed conversion rate (FCR) were recorded.

Another coetaneous trial was performed with litters of young rabbits from 17 to 44 d of age to study the effect of dietary supplementation with F, P and O oils on caecal activity around weaning. At 17 d, 120 kits from different mothers were randomly distributed into 12 litters of 10 kits (4 litters per diet), with free access to F, P or O diets. Females were separated from the litters and taken to the litter cages once daily in the morning for suckling for a short period until weaning at 28 d. After weaning, each litter was allocated to collective cages (50×80×32 mm high) and fed *ad libitum* with the same diet. Live weight and feed intake of lactating and growing litters were recorded at 21, 25, 28, 30 and 44 d of age.

Four rabbits per cage were slaughtered at 30 and 44 d of age (16 rabbits/d and treatment) between 20:00 and 22:00 h, and full gastro-intestinal tract (GIT) and caecum weights were recorded. After measuring the pH of caecal content (pH-meter GLP21, CRISON, Alella, Spain), aliquots of about 1 g were added to 3 mL of 2% sulphuric acid solution for ammonia nitrogen (NH_3) analysis, or with 2 mL of 2% orto-phosphoric acid to analyse the volatile fatty acids (VFA). Samples for VFA analysis were centrifuged at 10000×g for 10 min and the liquid phase was collected into Eppendorf vials of 1.5 mL. Finally, all samples were stored at –80°C until analysis. The remaining caecal content was stored at –20°C until DM analysis.

DM and NH_3 in caecum contents were respectively determined according to AOAC (2000), following procedures nº 934.01 and 973.49, respectively. Capillary gas chromatography was

used for VFA determination as follows: Samples were filtered through 0.45 μm cellulose syringe filters and 0.1 mL of an internal standard solution (0.4 g of 4-methyl-valeric acid diluted in 100 mL of deionised water) was added to 0.9 mL of filtrate sample. One μL from each sample was injected into a gas chromatograph (Fisons 8000 series, Milan, Italy) equipped with a split/splitless injector and a FID detector. VFA separation was done in a DB-FFAP capillary column (30 m×0.25 mm×0.25 μm of film thickness; J&W Scientific, USA). The carrier gas was N_2 at a constant pressure of 120 kPa. Both detector and injector temperatures were set at 245°C. The initial oven temperature was set at 115°C held for 5 min and increased to 230 at 8.5°C/min and finally maintained at that temperature for 10 min. VFA were identified by comparing their retention times with a standard 46975-U (Supelco®, PA, USA). VFA response factors obtained from this standard were finally used to calculate the VFA concentration in samples.

Statistical analysis

Data from the fattening trial were analysed using the GLM procedure from Statistical Analysis System (SAS, 2002), with a model that included the experimental oil (F,P,O), the α-tocopherol supplementation (0, 100), the Zn supplementation (0, 200) and their interactions as fixed effects, and the LW at 28 d of age as a covariate for DGW, DFI and FCR. Mortality rate during the growing period was analysed by the GENMOD procedure. Data on DWG of rabbits and DFI of litters around weaning were analysed using the PROC MIXED (SAS, 2002), according to a repeated measures design that took into account the variation between animals and covariation within them. Covariance structure was modelled for all the variables using compound symmetric (equal variances and correlations), unstructured (no assumptions regarding equal variances or correlations) and autoregressive structures (inter-animal random effect and a correlation matrix within animals that decrease with increasing lag between controls). Selection of covariance structure was done using the Schwarz Bayesian criterion (Littell *et al.,* 1998). The model included the diet (F, P and O), the control day (21, 25, 28 and 30 d) and their interaction as fixed effects. Random terms in the model included a permanent effect of each animal (p) and the error term (e), both assumed to have an average of zero, and variance σ^2_p and σ^2_e. Statistical analyses of the gastro-intestinal tract and caecal parameters at each age (33 and 44 d) were carried out according to a general linear model (GLM of SAS, 2002), where the model considered the diet as fixed effect (F, P and O), using the LW as covariate for GIT and caecum weights.

RESULTS AND DISCUSSION

Mortality was very high (31.8% on average; Table 2) but similar to that reported by other workers in trials conducted under the presence of ERE and non antimicrobial treatments (Blas *et al.,* 2010). However, no statistically significant differences between treatments were found.

Table 2: Mortality rate (%) of growing rabbits (28 to 63 d) according to oxidation state of dietary oil, α-tocopherol and Zn supplementation[1].

	Type of oil			α-tocopherol (ppm)		Zn (ppm)	
	Fresh	Peroxidised	Oxidised	0	100	0	200
No.	300	300	300	450	450	450	450
Mortality	29.7	31.7	34.0	31.3	32.2	29.1	34.4

[1] No effects were observed (*P*>0.05) for type of oil, α-tocopherol, Zn supplementation or any of their interactions.

The surviving rabbits were apparently healthy with normal growth rate and no clinical signs of the disease.

Table 3 shows the results obtained during the fattening trial with the 12 diets. Data from 286 dead animals during the experiment were removed from the statistical analyses, so 614 cases were included. No effect of the oxidation level of dietary oil on the daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) of animals throughout the growing period was observed, being on average 45.1 g/d, 107.6 g DM/d and 2.44, respectively. Only LW at 63 d was higher ($P<0.05$) in rabbits fed with diets supplemented with P oil (+32.3 and +41.3 g compared with the diets with F and O oils, respectively). However, the difference appears to be only due to values obtained for P and PZ diets (+4%) compared to those of F and OZ, and seemed to be related to the greater (although non significant) feed intake (+5-6%) observed with these diets.

Most works available in the literature on the effect of altered dietary oils were done with rats and mainly consider studies where peroxidised oils were overheated for a long time (until 25 d), obtaining oils with a high peroxide value (>200 meq O_2/kg), and frequently included at high level in diet (more than 100 g per kg feed). Under these conditions, most works reported a reduction in the feed intake, growth rate or conversion rate (Koch *et al.*, 2007) and an increase in physiological injures (Staprans *et al.*, 2005; Szarek *et al.,* 2006; Yuan *et al.*, 2007) in animals given the diet including overheated oil. However, when dietary oils were peroxidised close to commercial practice and included at a medium level in diets (80 to 100 g/kg), peroxide value did not increase too much and no effect on rat performance was observed (Eder and Kirchgessner, 1999; Quiles *et al.*, 2002; Totani and Ojiri, 2007). In fact, Totani and Ojiri (2007) even observed a tendency to increase feed consumption in rats fed with recovered frying oil compared to those given fresh oil. Moreover, no lesions were observed in the internal organs of turkeys subjected to prolonged feeding periods when diets with peroxide values of 5 meq O_2/kg feed (including 5% fat) were used, whereas the inclusion of fat with 50 meq O_2/kg caused morphological lesions in the liver (Szarek, *et al.,* 2006). Narasimhamurthy and Raina (1999), comparing the use of

Table 3: Effect of oxidation state of dietary oil, α-tocopherol and Zn supplementation on live weight (LW, g), daily weight gain (DWG, g/d), daily feed intake (DFI, g dry matter/d) and feed conversion rate (FCR) of growing rabbits (28 to 63 d).

Treatment								
Type of oil	α-tocopherol (ppm)	Zn (ppm)	No.	LW 28d	LW 63d	DFI	DWG	FCR
Fresh	0	0	53	569	2130	124	44.9	2.76
Fresh	0	200	50	586	2175	132	45.4	2.91
Fresh	100	0	61	591	2186	129	46.1	2.79
Fresh	100	200	47	601	2169	126	45.1	2.79
Peroxidised	0	0	57	594	2215	131	46.3	2.82
Peroxidised	0	200	53	591	2221	131	46.6	2.81
Peroxidised	100	0	46	590	2186	132	45.6	2.89
Peroxidised	100	200	49	580	2166	128	45.3	2.81
Oxidised	0	0	49	578	2165	129	45.8	2.82
Oxidised	0	200	47	590	2127	124	44.2	2.81
Oxidised	100	0	53	578	2154	128	45.0	2.83
Oxidised	100	200	49	584	2178	128	45.5	2.82

Continued Table 3.

Main effects	No.	LW 28d	LW 63d	DFI	DWG	FCR
Type of oil						
Fresh	211	592	2169[a]	128	45.4	2.81
Peroxidised	205	595	2199[b]	130	45.9	2.83
Oxidised	198	588	2167[a]	128	45.2	2.83
α-tocopherol						
0	309	595	2178	128	45.5	2.82
100	305	589	2178	129	45.5	2.83
Zn						
0	319	592	2181	129	45.6	2.82
200	295	592	2176	128	45.3	2.83
RSD	614	89.0	200	19.1	5.46	0.26
P-value						
Type of oil		NS	0.020	NS	NS	NS
α-tocopherol		NS	NS	NS	NS	NS
Zn		NS	NS	NS	NS	NS
Type of oil×α-tocopherol		NS	NS	NS	NS	NS
Type of oil×Zn		NS	NS	NS	NS	NS
α-tocopherol×Zn		NS	NS	NS	NS	NS
Type of oil×α-tocopherol×Zn		NS	NS	NS	NS	NS

[a,b] Means in a column with different superscripts differ at *P*<0.05.
NS: not significant (*P*>0.05)

different types of heated or fried vegetal oils included at 50 and 200 g/kg in the diets of rats, concluded that when the conditions employed for heating/frying were not drastic and the oils were not heat-abused, rats did not present any deleterious effect on growth rate, feed efficiency ratio and liver weight. Our results were in agreement with those obtained by Blas *et al.*, (2010) using a mixture of sunflower and olive recycled oils (p-anisidine value of 67) and seemed to confirm that recycled frying oils obtained under correct commercial practices can be used in rabbit nutrition.

According to Hamilton and Kirstein (2008), livestock are relatively resistant to oxidised fats with peroxide value below 100 meq O_2/kg oil (or reaching 4 meq O_2/kg in the complete feed), not affecting animal performance. In the present work, no disadvantageous effects of the use of peroxidised oils on the intake and growth performance of rabbits throughout the growing period were observed, coinciding with those results also obtained for broiler chickens (L'Estrange *et al.*, 1966; Cabel *et al.*, 1988), turkeys (Lea *et al.*, 1966) or pigs (Luci *et al.*, 2007). Kanner (2007) reported that lipid oxidation end-products absorbed from the gut into the blood circulation system seemed to act as injurious chemicals that trigger an inflammatory response in the liver, kidney, lung, circulatory system and the gut itself. However, if these products entered into circulation in minute amounts, they would be rapidly cleared by the reticulo-endothelial system, particularly in the liver. In fact, Tres *et al.* (2010a) found that 63 d old rabbits fed on the P diet during growing period showed a higher oxidation in liver and lower meat oxidative stability and α-tocopherol contents in plasma, liver and meat.

On the other hand, the addition of α-tocopherol and Zn to the diets had no effect on DFI, DWG and FCR of growing rabbits. According to Kanner (2007) and Hamilton and Kirstein (2008) this result could be related with the pre-stabilisation of fat with antioxidant before diet manufacture, and an adequate level of fat soluble vitamins and minerals from the raw materials and the vitamin/mineral mixture (which provides 20 and 60 ppm of α-tocopherol and Zn, respectively) included in the diets. However, while some works have reported a reduction in negative effects of highly-oxidised diets on animal performance when antioxidants were supplemented (Lin *et al.*, 1989; Bitam *et al.*, 2004), most works in the literature did not record any positive effect on growth performance from their additional supplementation (Schiavone *et al.*, 2010; Açikgöz *et al.*, 2011). These results reveal that when the usual stabilisation of oil and vitamin and mineral supplementation to meet animal requirements takes place, the additional inclusion of α-tocopherol and Zn seems to be unnecessary to ensure an adequate performance.

The oxidation level of supplemented sunflower oil did not affect the evolution of live weight of kits from 17 to 44 d of age, being on average 315, 358, 437, 526, 613 and 1271 g at 17, 21, 25, 28, 30 and 44 d, respectively. However, oil type affected DWG of young rabbits just after weaning (Figure 1), with kits fed on F and O diets showing higher growth than those receiving P diet from 28 to 30 d of age (54.5, 50.1 and 34.7 g/d, respectively; *P*<0.01). The lower DWG of rabbits fed on the diet supplemented with peroxidised oil after weaning seems to be occasional, and no effect on DFI and DWG during the whole period was observed, being on average 77 g DM/d and 46 g/d, respectively. In weaning pigs, Yuan *et al.* (2007) observed a depression in growth performance due to oxidative stress, with reduced activities of anti-oxidative enzymes in plasma and liver that could promote biological injures with diets including 5% oil. However, seriously oxidised fish oil (786.5 meq O_2/kg) heated 60 h at 37°C and with addition of H_2O_2, $FeSO_4$ and $CuSO_4$, was used in this work. In any case, oxidation state of dietary fat could be relevant for rabbit health, as it could affect adequate initial gut development when the solid intake of kits is increasing quickly (Soler *et al.*, 2005). This fact may be especially considered with peroxidised oils, as hydroperoxides could injure the intestinal mucosa and have a role in molecular events related with degenerative intestinal disorders (Hamilton and Kirstein, 2008).
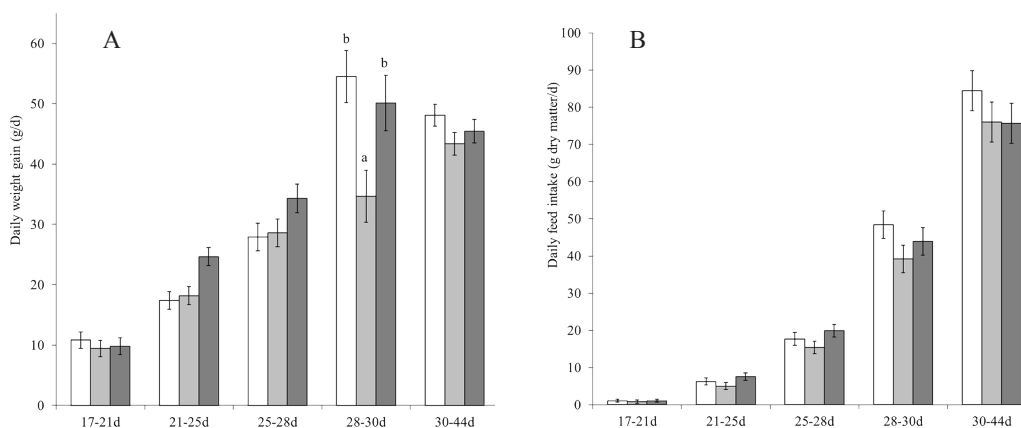
Figure 1: Evolution of A) daily weight gain and B) daily feed intake of young rabbits (17 to 44 d; P_{age}<0.001) in function of the dietary sunflower oil used: fresh (□), peroxidised (▨) and highly oxidised (▨).
No.=120; [a,b] Means with different superscripts differ at *P*<0.05. Bars indicate standard error..

Table 4 shows the effect of the lipid oxidation state of dietary added oil on GIT and caecum weight and some caecal parameters of young rabbits at 30 and 44 d of age. Young rabbits fed with the P diet showed lower DM percentage in caecum content at 30 d of age (–9.5%; $P<0.05$) than those receiving F and O diets. The lower caecal DM content of kits receiving the peroxidised added oil could be related to the lower feed intake values (–12%, not significant) observed just after weaning. The degree of feed acceptability in the young rabbit could play an important role around weaning, where transition from milk to solid feeding takes place. Although the usual secondary oxidation products in oils heated at high temperatures (O oil) are relatively unreactive (Hamilton and Kirstein, 2008), hydroperoxides present in oils heated at moderate temperatures are highly reactive, which could affect the diet's acceptability for the animals. In fact, Yuan et al., (2007) observed a reduction of voluntary feed intake during the first 2 wk after weaning (–12%; $P<0.05$) in piglets fed with a diet including 5% of a highly peroxidised oil (786 meq O_2/kg), but feed intake and daily weight gain of pigs were not affected thereafter.

Table 4: Full gastro-intestinal tract (GIT) and caecum weights (% live weight), and caecal parameters of rabbits at 30 and 44 d of age in function of the dietary sunflower oil used: fresh, peroxidised and highly oxidised.

		No.	Fresh	Peroxidised	Oxidised	SEM	P-value
Full GIT weight	30 d	16	26.5	27.1	26.6	0.62	NS
	44 d	15	28.1	27.2	29.1	0.65	NS
Full caecum weight	30 d	16	8.5	9.0	8.5	0.54	NS
	44 d	15	9.6	9.3	9.4	0.32	NS
Caecal parameters							
Dry matter (%)	30 d	15	21.2[b]	18.8[1a]	20.2[b]	0.46	0.015
	44 d	15	18.2	18.1	18.0[2]	0.48	NS
pH	30 d	15	5.94	6.03[1]	5.78	0.07	NS
	44 d	15	5.76	5.88	5.83	0.08	NS
NH$_3$ (mmol/L)[3]	30 d	16	9.02[b]	7.87[ab]	5.58[a]	0.92	0.044
	44 d	15	10.3	8.66	9.87	0.95	NS
VFA (mmol/L)[3]	30 d	16	138	149	141	10.9	NS
	44 d	15	142[ab]	131[a]	178[b]	12.1	0.032
Acetic acid (mmol/L)	30 d	16	115	127	120	9.7	NS
	44 d	15	121[ab]	110[a]	154[b]	10.8	0.026
Propionic acid (mmol/L)	30 d	16	9.21	9.23	7.53	0.76	NS
	44 d	15	6.76	6.53	8.76[2]	0.84	NS
Butyric acid (mmol/L)	30 d	16	13.0	11.0	12.0	1.24	NS
	44 d	15	13.5	12.4	14.2	1.37	NS
Acetic/propionic rate	30 d	16	13.3	14.9	17.1	1.40	NS
	44 d	15	18.4	19.2	18.4[2]	1.56	NS

[1] No.=16. [2] No.=14. [3] NH$_3$: Ammonia nitrogen in caecal content, VFA: Total volatile fatty acids concentration in caecal content.
[a,b] Means in a row with different superscripts differ at $P<0.05$.
NS: not significant ($P>0.05$).

On the other hand, caecum of young rabbits fed with the O diet presented a lower NH_3 content at 30 d of age compared to those given F diet (–38%; *P*<0.05) and higher total VFA concentration at 44 d than those fed with P diet (+36%; *P*<0.05). Although the main volatile fatty acid values in caecum (acetic, propionic and butyric) were the highest for those fed with the O diet, significant differences were only found for acetic acid content in caecum (+34% compared to P diet; *P*<0.05).

Both lower NH_3 content and higher VFA concentration with O diet indicate a higher microbiological activity in caecum. Vegetable oils subjected to overheating conditions for a long time lead to a reduction in PUFA (Bou *et al.*, 2005; Tres *et al.*, 2010b; Blas *et al.*, 2010) as oxidation is mainly addressed to double bonds, but also in an increase of volatile compounds that derived from hydroperoxy or alcoxy radical breakdown. In ruminants, vegetal oil addition has frequently been related to decreases in fibre digestibility due to the negative effect of PUFA and radicals on ruminal microbial population (Ikwnegby and Sutton, 1982; Chalupa *et al.*, 1984). The amount of fat reaching the rabbit caecum is low, but its chemical composition or alteration might affect caecal fermentation. In fact, Fernández *et al.* (1994) described a reduction in energy and fibre apparent digestibility coefficients in weaned rabbits when unsaturated oils were added to diet. Similarly to the present work, Falção-e-Cunha *et al.* (2004) described a tendency to reduce the total VFA and acetic acid concentration, as well as cellulose activity, in the caecum of growing rabbits when a 6% sunflower oil was included in the diet.

In conclusion, the use of oxidised oils with a ρ-anisidine value of 125 in livestock feeds did not affect the performance and caecum status of growing rabbits. The use of peroxidised oils with a peroxide value of 83 meq O_2/kg oil could affect the degree of feed acceptability by the young rabbits, but this effect was discrete and the short growing period is not enough to affect the health and growth of rabbits. Therefore, and although many questions are still open and further research will be necessary, oxidised oils could be considered as a possible energy source for rabbit nutrition.

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