EFFECT OF DIETARY FAT QUALITY ON THE PERFORMANCE AND HEALTH OF FATTENING RABBITS

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ABSTRACT

Three experiments were performed with 786 weaned rabbits (28-day old) in order to evaluate the effects of feeding recycled fatty materials on the performance (690 rabbits) and health status (96 rabbits) during the fattening period. Six experimental feeds for rabbits were formulated including 3% of fats with low (L) or high (H) levels of *trans* fatty acids, polycyclic aromatic hydrocarbons (PAHs) or lipid oxidation. Weight gain and feed intake were recorded between 28 and 56 days and between 57 and 63 days. In 63-day old animals, the caecal ambient was assessed by measuring pH, NH_3 and short chain fatty acids (SCFA) in caecal content, and the hepatic and renal functions by determining gamma glutamyl transferase (GGT), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), urea and creatinine on serum samples. Because of Epizootic Rabbit Enteropathy, a very high mortality rate was recorded (varying between trials from 31% to 60%), with no statistically significant differences between L and H treatments in any experiment. No statistically significant differences in performance were linked to the dietary levels of *trans* fatty acids (body weight gain and feed intake in this experiment averaged 46.8 g/day and 121 g dry matter/day, respectively), PAHs (45.1 g/day and 107 g dry matter/day) or lipid oxidation (45.1 g/day and 108 g dry matter/day). Similarly, blood levels of the hepatic or renal function markers were not affected by the dietary levels of *trans* fatty acids (GGT, GOT, GPT, ALP, urea and creatinine averaged 7.29 IU/l, 64.7 IU/l, 65.4 IU/l, 156 IU/l, 20.7 mg/dl and 0.82 mg/dl, respectively), PAHs (6.43 IU/l, 76.0 IU/l, 64.0 IU/l, 148 IU/l, 19.7 mg/dl and 0.73 mg/dl) or lipid oxidation (6.32 IU/l, 69.3 IU/l, 59.4 IU/l, 155 IU/l, 17.5 mg/dl and 0.79 mg/dl). However, some dietary effects on caecal ambient were detected. Thus, a higher level of *trans* fatty acids (and saturated fatty acids) was associated with lower SCFA concentration in caecal content (164 vs. 215 mmol/l, $P<0.01$). On the other hand, caecal NH_3 concentration was higher in rabbits receiving the diet high in PAHs (11.7 vs. 5.56 mmol/l, $P=0.04$). No effects of dietary lipid oxidation on caecal parameters were detected.

Key words: Fattening rabbits, Recycled fatty materials, Trans fatty acids, Polycyclic aromatic hydrocarbons, Lipid oxidation.

INTRODUCTION

The use of fat in animal feed manufacturing has increased parallel to the improvements in the productive potential of the several species. In practical situations, it is not likely to design programs of feeding for high productive potential animals without adding fat to the diet.

In the European market numerous types of fats exist and its use in feed varies from country to country based on its availability and on the relative price respect to other energy sources. The presence of fats/oils in rabbit feed brings several important nutritional and technological advantages (Fernández-Carmona *et al.*, 2000), but it is necessary to know that they have components (inherent to their composition or derivative from the manufacturing process, alteration or accidental contamination) that can modify their nutritious properties, therefore its use as a main energy source in animal nutrition. The presence of degradations products and contaminants could produce a decrease of the nutritional value of fat sources and affect performance or health status of the animals.

Very few information exists on the effect of inclusion of fats recycled from the food chain in animal nutrition. The results derived from Project FFS (Bondioli *et al.*, 2007) show that fats and oils commonly included in feeds vary enormously on their composition and can be altered or contain undesirable substances. There are numerous scientific papers focusing on the study of the origin of these undesirable compounds or their presence in different ingredients or foods. In parallel, studies have been made by epidemiologists to evaluate the level of tolerance and the consequences of its consumption by people. Nevertheless studies focusing on the effect of consumption of these compounds by animals are scarce. It should be clarified if the consumption of altered or contaminated fats during the productive period could hit their performance, health and well-being.

The objective of this study is the evaluation of the effects of feeding recycled fatty materials differing in their levels of fatty acid isomers or polycyclic aromatic hydrocarbons (PAHs) or oxidation on the performance and health status of fattening rabbits.

MATERIALS AND METHODS

Six experimental feeds for fattening rabbits were formulated according to their nutritional needs (De Blas and Mateos, 1998), with 97% of an identical basal mix and including 3% of the appropriate fat. The included fats were:

- "*Trans* fatty acids" experiment (T): a palm fatty acid distillate and its corresponding hydrogenated palm fatty acid distillate, respectively with low and high trans fatty acid content (0.65% vs. 12.4%)
- "PAHs" experiment (P): two acid oils from chemical refining of olive oil and olive pomace oil, respectively with low and high PAHs content (<18 ng/g oil vs. 5290 ng/g oil)
- "Lipid oxidation" experiment (O): a vegetable oil, fresh or recycled after using in a commercial frying process and then heated at 165-170ºC during 8 hours, respectively with low and high level of lipid oxidation (levels of polymers: 0.35% vs. 6.61%; p-anisidine values: 2.74 vs. 67.43)

Consequently, rabbit feeds obtained for the three different experiments had low (L) or high (H) level of *trans* fatty acids, PAHs or lipid oxidation, respectively.

Feed samples were taken for analysing dry matter, ashes, crude protein, ether extract and crude fibre following the AOAC (1995) procedures, and detergent fibres according Van Soest *et al.* (1991).

The composition and the average nutrient analyses of experimental feeds are shown in Table 1.

Table 1: Ingredients (%) and average nutrient composition (%) of the experimental diets

Ingredient		Composition *as feed basis*	
Barley	10.0	Dry matter	89.5
Beet pulp	30.0	Ash	8.5
Sunflower meal	20.0	Crude protein	13.1
Alfalfa hay	34.0	Ether extract	4.2
Added fatty material	3.0	Crude fibre	20.1
HCl L-lysine	0.35	Neutral detergent fibre	35.4
DL-methionine	0.2	Acid detergent fibre	22.7
L-threonine	0.15	Acid detergent lignin	4.5
Dicalcium phosphate	1.3		
Salt	0.5		
Vitamin and mineral premix[1]	0.5		

[1]Composition of vitamin and mineral premix (1 kg of feed contained): Vitamin A: 8375 UI; Vitamin D3: 750 UI; Vitamin E: 20 mg; Vitamin K_3: 1 mg; Vitamin B_1: 1 mg; Vitamin B_2: 2 mg; Vitamin B_6: 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; BHA+Ethoxiquin: 4 mg

A total of 690 weaned rabbits (28-day old) were used in three growth trials with L and H diets (230 animals/trial, 115 animals/diet); animals were housed in individual cages, with water and feed

provided *ad libitum*, until 63-day old; weight gain, feed intake and feed efficiency were recorded between 28 and 56 days and between 57 and 63 days. Another trial was performed with 96 weaned rabbits (6 diets, 16 animals per diet) in order to test the possible effects of feeds on the caecal ambient and on the hepatic and renal functions; animals were allocated in collective cages and fed *ad libitum* on the experimental diets; at slaughtering, at 63-day old, blood and caecal content samples were taken from a total of 48 healthy surviving animals (8 animals per diet). Housing, husbandry and slaughtering conditions agreed to current European Union guidelines and all trials were subject to agreement of Animal Protocol Review Committee of the UPV.

After measuring the pH of caecal content (pH-meter GLP21, CRISON, Alella, Spain), aliquots of about 1 g of were weighted and added with 3 ml of 2% sulphuric acid solution or 2 ml of 2% orto-phosphoric acid for analysing NH_3 and short chain fatty acids (SCFA), respectively. Samples for SCFA analysis were centrifuged at 10000 *g* during 10 minutes and the liquid phase was collected into eppendorfs vials of 0.5 ml. Finally, all samples were stored at –80ºC until analysis. The rest of caecal content was stored at –20ºC until dry matter analysis. Dry matter and NH_3 in caecal content was determined according the AOAC (1995) procedures. For SCFA analysis, samples were previously filtered through a cellulose filter (0.45), and 250 µl of them were transferred to the injection vials. Two µl from each sample were injected into the gas chromatograph (FISONS 8000 series, Milan, Italy) equipped with an automatic injector AS800. The column used was a BD-FFAP 30x0.25x0.25 mm. The temperature of the injector and the detector were maintained at 220ºC and 225ºC, respectively. Acetic, propionic, butyric, iso-butyric, valeric, iso-valeric, caproic and heptanoic acids were determined. After clotting at ambient temperature, serum was obtained by centrifugation at 1500 *g* during 10 minutes, being stored in eppendorfs vials of 2 ml at –80ºC until analysis. Serum analyses for assessing hepatic and renal functions were performed by means of an automatic analyser (Vitros 900, Johnson and Johnson, Rochester, NY, USA). The analytical determinations performed were gamma glutamyl transferase (GGT), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), urea and creatinine.

Data from each experiment were analysed through a one-way ANOVA using the GLM procedure of using SAS System® Software (SAS, 2002).

RESULTS AND DISCUSION

Because of Epizootic Rabbit Enteropathy, a very high mortality rate was recorded (60%, 31% and 33% in T, P and O trials respectively), but no statistically significant differences between L and H treatments of each trial were found. In fact only rabbits apparently healthy (normal growth rate, no clinical signs) were used for final analyses. As a matter of fact, most of 63-day old surviving animals were healthy.

As showed in Table 2, no statistically significant differences in growth performance and in blood levels of the hepatic or renal function markers were linked to the dietary levels of *trans* fatty acids, PAHs or lipid oxidation. However, some dietary effects on caecal ambient were detected. Thus, higher level of *trans* fatty acids was associated with lower total SCFA concentration in caecal content, with minor changes in the molar proportions of acetic and butyric acids (the former increased from 88.0% to 89.4%, SEM=0.4, P=0.03; the later decreased from 8.48% to 6.99%, SEM=0.43, P=0.03), what could indicate some impairment of microbial activity; it must be taken into account that hydrogenated palm fatty acid distillate is richer not only in *trans* fatty acids but also in saturated fatty acids than palm fatty acid distillate (85% vs. 54%) and then it is not possible to separate the effect of both variations. The values of total SCFA concentration in the current study were clearly higher than those usually referred in the literature (40-80 mmol/l), probably because in the current study the concentration in the samples injected into the gas chromatograph was multiplied by a dilution factor (F_i) to take into account the initial addition of 2 ml of 2% orto-phosphoric acid to samples of caecal content [$F_i = (W_i*(1-DM_i)+2)/W_i*(1-DM_i)$, being W_i and DM_i the weight (g) and the dry matter content (g/g) of the sample, respectively]. On the other hand, caecal NH_3 concentration was higher in rabbits receiving the diet high in PAHs, meaning worse caecal ambient and more risk of disbiosis in

the caecal microbial ecosystem; that could be connected with the lower digestibility of this diet (Cervera *et al*., 2007), promoting greater protein ileal flow and higher proteolytic activity by caecal bacteria; the higher, though not statistically significant, urea concentration in blood would be associated to this fact. No effects of dietary lipid oxidation on caecal parameters were detected.

Table 2: Growth performance, caecal parameters and hepatic or renal function markers of rabbits fed on diets low (L) or high (H) in *trans* fatty acids, PAHs and lipid oxidation

	trans fatty acids experiment				PAHs experiment				lipid oxidation experiment			
	L	H	SE	*Prob.*	L	H	SE	*Prob.*	L	H	SE	*Prob.*
Body weight gain (g/day):												
28 to 56 days	48.0	47.2	0.8	0.46	46.1	46.5	0.8	0.66	46.1	46.2	0.9	0.95
56 to 63 days	43.4	43.4	1.2	0.98	39.8	41.0	1.0	0.35	41.1	40.3	1.2	0.58
Feed intake (g DM/day):												
28 to 56 days	113	113	2	0.93	100	102	2	0.57	100	100	2	0.97
56 to 63 days	152	150	3	0.55	132	134	2	0.40	139	139	3	0.86
Feed efficiency:												
28 to 56 days	2.36	2.40	0.04	0.37	2.18	2.19	0.03	0.75	2.18	2.16	0.03	0.56
56 to 63 days	3.62	3.66	0.12	0.76	3.42	3.33	0.07	0.32	3.47	3.60	0.09	0.31
Caecal parameters:												
pH	5.75	5.87	0.07	0.21	5.53	5.69	0.07	0.12	6.02	6.03	0.11	0.94
NH_3 (mmol/l)	7.01	3.63	1.39	0.11	5.56	11.7	1.92	0.04	5.16	4.76	1.21	0.82
Total SCFA (mmol/l)	215	164	10	<0.01	251	202	24	0.18	244	215	30	0.50
Hepatic function (IU/l):												
GGT	7.14	7.43	0.32	0.54	6.56	6.29	0.52	0.72	6.63	6.00	0.34	0.22
GOT	62.3	67.0	2.6	0.22	74.9	77.0	3.9	0.71	67.2	71.3	2.2	0.22
GPT	62.1	68.6	2.8	0.13	67.7	60.3	2.8	0.12	57.9	60.8	2.7	0.46
ALP	158	154	10	0.80	142	153	7	0.25	147	162	9	0.24
Renal function (mg/dl):												
Urea	22.3	19.0	1.2	0.07	17.6	21.7	1.9	0.15	17.2	17.8	0.7	0.54
Creatinine	0.80	0.83	0.02	0.34	0.76	0.69	0.03	0.11	0.75	0.82	0.03	0.09

SE: standard error of means; GGT: gamma glutamyl transferase; GOT: glutamic oxaloacetic transaminase; GPT: glutamic pyruvic transaminase; ALP: alkaline phosphatase; SCFA: short chain fatty acids

CONCLUSIONS

The inclusion of recycled fatty materials with high levels of *trans* fatty acids, PAHs or lipid oxidation in rabbit feeds seems not impair growth performance or hepatic and renal functions, but may cause some unfavourable changes in the caecal ambient.

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