Project nº: *FOOD-CT-2004-07020*

Project acronym: *FEEDING FATS SAFETY*

Project title: *Quality and safety of feeding fats obtained from waste or by-products from the food chain*

Instrument: *Specific Targeted Research Project (STREP)*

Thematic Priority: *FOOD QUALITY AND SAFETY*

Deliverable 16

Final Report and Technical Implementation Plan

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Project Coordinator: *RAFAEL CODONY*
Project coordinator organisation: *UNIVERSITY OF BARCELONA*

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1.PROJECT EXECUTION

1.1.Project objectives

The research project *Feeding Fats Safety* was devised to respond to the needs of feedstuff and meat producers to increase the safety of fats included in animal feed. Thus, the objective of the project is to provide information on the quantitative effects of several contaminants (dioxin, PCBs, PAHs, PBDEs) and lipid degradation products (oxidation, isomerization and polimerization) in feed fats on the quality of meat, as well as data on the repercussions of these compounds on animal health and productive parameters, and finally on human health. We proposed to assess the extent of the risks of including a number of fat sources obtained from co- or by-products from the food chain, such as animal fats, fish oils, acid oils from refining processes, in the manufacture of animal feed. The lack of product standardization could entail a risk in the use of these fats. Therefore, one of the aims of the project is to establish a classification and characterization protocol for fats included in animal feed production. To date, this use of fats has not been strictly regulated and producers have included in feeds various low quality, low-cost fats, sometimes derived from degraded or contaminated waste materials. The lack of regulations in this secor also hinders the development and commercialization of special fatty products and the use of several natural by-products/co-products from oil refining. The proper use of fat products is also of relevance to the environment as it could lead to a reduction in the amount of valuable materials to be disposed of. According to this criteria, the objectives of the project were:

- To improve the standardization of these fats for feed production purposes. More data on the composition, degradation and contamination of these recycled fat materials would contribute to improve the standardization of these materials, and to the development of a regulation and control policy.
- To examine the effects of these fats in animal production when they present high levels of undesirable characteristics.
- To approximate the extent of the transfer of contaminants from these fats to meat.
- To propose suitable analytical controls to assess the quality and safety of these fats in meat production.
- To propose modifications, when required, to improve the quality of these fats and to reduce their levels of contamination.
- To study the socio-economic impact of the inclusion of these fats in feeds compared with alternative uses.
- To increase consumer awareness of food production sectors that use these fats and, consequently, improve consumer confidence in products of animal origin.

The strategic impact of the results is related mainly to consumer health protection, but also to the improvement of the most critical step in the chain of obtention of foods from animal origin, namely the feedstuff quality control. Recent cases of a lack of safety of some foods of animal origin has been associated with a failure in this step, and the consumer confidence must be recovered by reinforcing the quality of feed production. In addition, the results of this project can help European fat and feedstuff producers to improve the quality of their production systems and increase their competitiveness with non member countries. The results expected are:

- To help European institutions to improve the legal regulation of feed fats quality;
- To provide oil and fat producers with improved information about the characteristics (chemical composition, usual degradation and contamination levels) of a wide array of fat by- or co-products;
- To provide feed manufacturers with a set of guidelines for a better use of feed fats, taking profit from recycled materials coming from the food chain;
- To promote the use of feeds including fat co- and by-produts by meat producers, ensuring the highest standards of quality and safety, and thereby minimizing the public health risk;
- To improve the analytical control of feed fats, feedstuff, and meat products, by defining and recommending the most useful control parameters;
- To improve the level of information and the level of confidence of the European consumer in foods from animal origin.

1.2.Contractors involved

Resources needed to achieve our objectives have been covered by the experience and capability of the partners constituting the consortium. The research efforts needed to achieve the objectives of the project are assured by the wide variety of disciplines covered by the different teams. All these groups have very powerful resources, since they are integrated in high Departments, Institutes or Companies, having a large experience in research projects in different aspects of feed and food quality and safety. Thus, the scientific and technological objectives of the project have been suitably covered by tasks corresponding to every team and by the integration activities developed all along the project. Vertical integration was ensured, since the nine teams are selected to cover all areas of study all along the meat production chain, from fat production and feed manufacture until meat supply for human consumption. Horizontal integration was also assured, since the seven teams involved are specialised in different scientific and technological areas: veterinary, chemical, biochemistry, analysis and quality control, environment, nutrition sciences and fat and feed production technology.

Contractors involved were the following, under the coordination of the University of Barcelona:

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1.3.Work plan and execution

The work plan of the project includes two parts:

PART I

One hundred and twenty-four feed fat samples were collected from various factories belonging to 10 European countries. They corresponded to nine types or "categories", according to their respective source and processing technology, and all of them are or have been usually included in feed formulation. Sampling procedure was standardized and each factory filled a form corresponding to each sample, including the maximum information. Samples collected were: 25 acid oils from chemical refining (AOCHE); 16 acid oils from physical refining (AOPHY); 8 lecithines (LECI); 8 recycled cooking oils (RECY); 36 animal fats (ANFA); 2 oils extracted from exhausted bleaching earths (EBE); 9 fish oils (FISH); 6 hydrogenated by-products (HYBY); 3 fatty acid calcium soaps (FACS); and several fats that were included in a miscellaneous group (MIX) since they did not correspond to the previous categories.

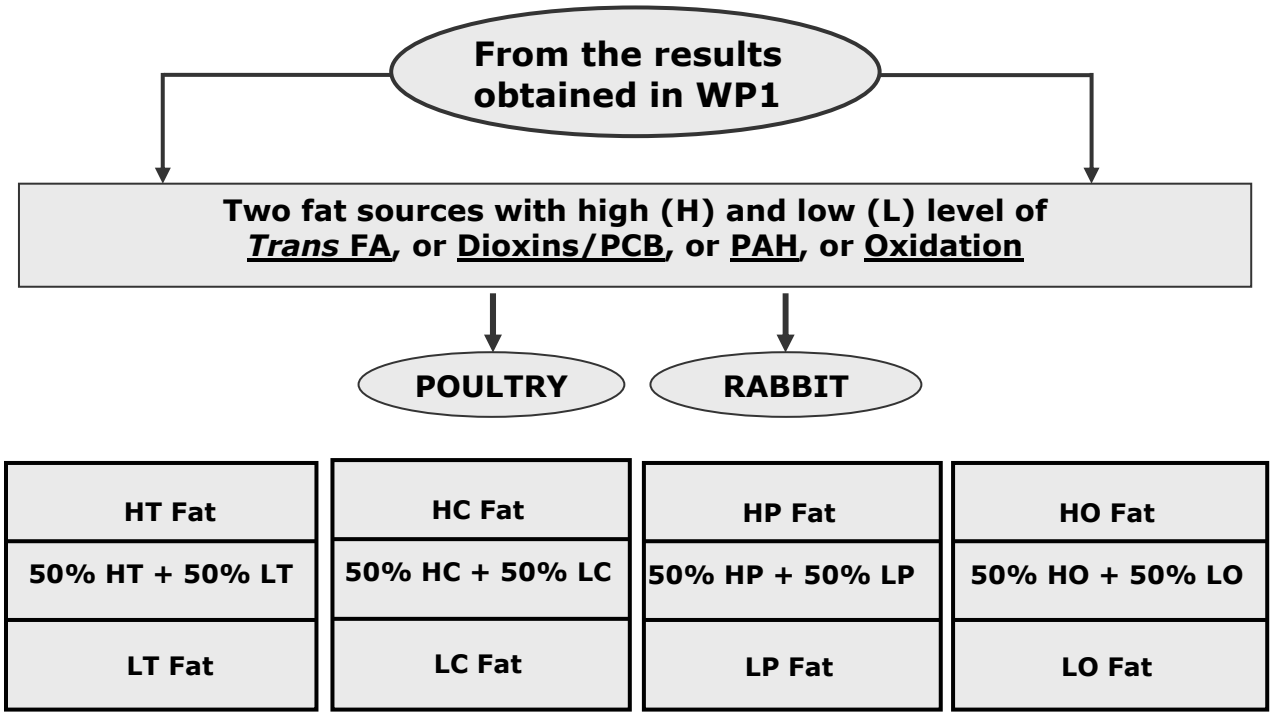
Analytical parameters applied for the characterization of these fat samples were: a) Composition, including moisture, acid value, mono- and di-glicerydes, FA composition, *trans* fatty acids (TFAs), conjugated linoleic acid isomers (CLA), sterols, and tocopherol/tocotrienol content; b) Degradation, including sterol oxidation products (SOPs), oxidated FA, triglyceride polymers, peroxide value, TBA value, *p*-anisidine

value, and oxidative stability (by a colorimetric induced method-FOX); c) Contaminants' content, including dioxins (PCCD/PCDFs), DL-PCBs, PBDEs and PAHs.

Moreover, the Fourier-transformed infrared spectroscopy (FTIR) was applied to the samples in order to propose a fat classification system according to the corresponding spectra obtained.

PART II

We designed a series of four animal trials to test the effects (in poultry and rabbit) of selected fat by- and co-products with low and high contents of TFAs, oxidation products and the above mentioned contaminants. These trials sought to assess: a) the harmful effects of these fats included in feeds for poultry and rabbit; b) the effects of these fats on the lipid composition of meat and other tissues and fluids; c) the presence of TFAs, CLA, FA oxidation and sterol oxidation products in these tissues; d) the rate of transfer of contaminants from feed to meat and other tissues. The four trials, with three dietary treatments per trial, were:



T = *trans* FA isomers **C** = dioxins, PCB & PBDE **P** = PAH **O** = oxidation level

4 TRIALS x 3 treatments x 8 replicates = 4 x 24 = TOTAL 96 RABBIT samples
TOTAL 96 POULTRY samples

192 MEAT samples/192 LIVER samples/192 PLASMA samples (+bile, excreta, urine)

As a consequence of the experimental design, we had to select 2 different oils or fats for each trial, containing "high" and "low" levels of the parameter to be assayed in each trial. The selection of the most suitable fat materials for the trials was made according to the following criteria: a) levels of the factor to be evaluated (*trans* FA, Dioxins/PCB, PAH, and Oxidation) should be among those found in samples collected and characterised inside WP1; b) the two fats selected for a trial (high and low level, respectively) should be as similar as possible in their FA composition, in order to avoid interferent effects of FA in the parameters determined; and c) the two fats selected should also be similar in other characteristics that could be potentially interferent in the assessment of effects, mainly contaminant levels. According to these criteria, and taking into account the results obtained inside WP1, a palm fatty acid distillate was chosen as representative of "low" *trans* FA content, respect to a hydrogenated palm fatty acid distillate which would be representative of "high" *trans* FA content. In this case, obviously, the second criteria (similar FA composition) could not be considered. For the Dioxin trial, two fish oils were selected among those analysed inside WP1, one showing very low levels of dioxins-PCBs and another with quite high levels of these contaminants. For the PAH trial, taking into account the PAH levels observed in WP1 for different fats and trying to have similar fatty acid profile, we selected two acid oils from chemical refining, one from olive oil (with very low levels of these contaminants) and another from pomace olive oil (with quite high levels of these contaminants). Finally, for the oxidation trial we include a sunflower-olive oil blend (70:30, v/v), before and after to be used in a commercial frying process. Finally, "intermediate" fats for each trial were prepared, by blending (1:1, v/v) the corresponding "high" and "low" fats.

A fifth trial ("Spike trial") was specifically conducted with poultry to assess the rate of transfer from feeds to meat and other animal tissues of some selected contaminants (which had been chosen according to their higher safety interest), added to the feed at 4 different levels.

The selected organic contaminants added to chicken feeds were:

-PAHs: phenanthrene (Phe), fluoranthene (Fluo), chrysene (Chrys), benzo[e]pyrene (BeP) and benzo[a]pyrene (BaP),

-PBDEs: BDE 47 and BDE 153

-Dioxin analytes: 17 toxic PCDD/Fs + 12 DL-PCBs.

The selection of the different spike levels was based on the maximum levels of individual compounds found in the oils/fats characterized during the WP1 exercise, taking into account the usual addition percentages of oils/fats to chicken feed. As an example, the maximum individual PAH concentration determined in oils was 10 µg/g; assuming a 6% content of oil in the final chicken feed, the corresponding spike level in feed should be 600 ng/g. The four treatment levels were then defined as follow:

- Level I (DP1): Control (background level in the oil)
- Level II (DP2): Half of the maximum contamination found in WP1 oils
- Level III (DP3): The maximum contamination found in WP1 oils
- Level IV (DP4): Twice the maximum contamination found in WP1 oils

However, due to the extremely low levels of PBDEs determined in oils/fats (compounds detected only in fish oils at a maximum level of 10 ng/g each, leading to levels as low as 0.6 ng/g in final feed), it has been decided to increase by a factor of ten this maximum level.

In the case of PCDD/Fs and DL-PCBs there are already maximum levels established by the EU. The maximum levels for "Compound feedingstuffs, with the exception of feed for animals, pet foods and feed for fish" established at the Commission Directive 2006/13/EC of 3 February 2006 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs are 0.75 ng WHO-PCDD/F-TEQ/kg and 1,5 ng WHO-PCDD/F-PCB-TEQ/kg. Taking into account that there is a separate maximum level for PCDD/Fs but not for DL-PCBs, the most useful was to consider, as a maximum levels for the experiment: 0.75 ng WHO- TEQ/kg of feed for PCDD/Fs and 0.75 ng WHO- TEQ/kg of feed for DL-PCBs, thus the summatory will give the maximum of 1.5 for all the compounds. Finally, the expected contents of individual selected compounds in chicken spiked feed are:

- Level I (DP1): Control (background)
- Level II (DP2): 300 ng/g PAH + 3 ng/g PBDE + 0.38 pg/g WHO-TEQ PCDD/Fs + 0.38 pg/g WHO-TEQ DL-PCBs
- Level III (DP3): 600 ng/g PAH + 6 ng/g PBDE + 0.75 pg/g WHO-TEQ PCDD/Fs + 0.75 pg/g WHO-TEQ DL-PCBs
- Level IV (DP4): 1200 ng/g PAH + 12 ng/g PBDE + 1.5 pg/g WHO-TEQ PCDD/Fs + 1.5 pg/g WHO-TEQ DL-PCBs

A part from these two big parts of the project, constituting Work Packages 1, 2, 3, 4 and 5, the project included other complementary studies:

- A "Consumer opinion study" was designed to assess the opinion of the European consumer about the use of these fat co- and by-produtcs in feeds for animal production. Their opinion about the interest of the project was also requested.

- A "Socio-economic study" was designed to assess the potential impact of the use in feeds of the total amounts of these fats produced in Europe, analysing the balance

between the benefits and the risks of their use in feeds and taking into account other alternative uses.

1.4.Results

1.4.1.Chemical composition and degradation of feed fats

Regarding the characterization of feed fats collected, corresponding to categories mentioned above, the main results can be summarised as follows. We give only a brief overview about the main parameters that showed more characteristic values for each feed fat category.

The 25 acid oils from chemical refining analized (AOCHE) were mainly constituted by a mixture of free fatty acids and triglycerides, with a high “Acid value”. Fatty acid composition varied according to the oil or fat refined (olive, seed, animal, fish). Low “Peroxide Values” were found but secondary oxidation (measured as “p-anisidine value”) was above the other co-/by-product groups analyzed, except the fish oils.

The 16 acid oils from physical refining (AOPHY) were mainly constituted by free fatty acids (“Acid values” even higher than those corresponding to AOCHE) and non saponifiable compounds, particularly tocopherols and tocotrienols. Similar to AOCHE, AOPHY showed low “Peroxide Values” but high secondary oxidation product contents.

The 9 lechitins analyzed showed a very high % polyunsaturated fatty acids, since they are constituted mainly by phospolipids and triglycerides. Moreover, high and variable Acid Values were found and high secondary oxidation values. Very high sterol content was also found in these LECI samples. Moisture was not well standardized.

Thirty-six animal fats (ANFA) were analyzed. The fats differed considerably in fatty acid composition and in TFA content, depending on the animal source. TFA and CLA contents were good markers of the presence of ruminant fats. ANFA showed in general very low acid and oxidation values.

The 9 fish oils (FISH) analyzed showed the particular fatty acid composition of this type of source. However, they showed a certain variability in the percentage of n-3 polyunsaturated FA. A certain level of CLA was also found, which had a degradation

origin. The level of degradation in these FISH samples was confirmed by the high p-Anisidine values (mean =73) and polymer contents (mean =2%), which were among the highest found in all fat catagories. In contrast, low Acid Values were observed in fish oils.

Neither RECY nor EBE are not used nowadays as feed ingredients, because it is difficult to standardize their quality and decrease the risks involved in their use. Eight RECY and 3 EBE samples were analyzed. The fatty acid composition and other main components of these oils were variable since their origin is always mixed. EBE were characterized by a higher Acid Value than RECY, while RECY showed higher oxidation values. Both EBE and RECY showed the highest polymer content (mean, 9%) among all the fat categories. Both types of oil showed intermediate but consistent CLA contents, originated mainly by degradation.

HYBY and FACS show particular composition characteristics, that make them useful only for certain animal feeds (i.e. ruminants). FACS (3 samples) showed a high percentage of saturated FA, which can be atributed to the fact that they usually come from tropical oil sources (palm, plam kernel and coconut). HYBY, intended as partially hydrogenated oils, (6 samples) showed an even higher percentage of these fatty acids, as a result of hydrogenation. HYBY were also high in free fatty acids since palm fatty acid distillates are usually the raw material used. Another relevant difference was that HYBY are much richer in tocopherols and tocotrienols but much less rich in sterols than FACS. As a result of hydrogenation, HYBY also showed the highest contents of TFA, although the total amount was variable (1,9-9,4 %).

The application of the FTIR spectroscopy to the analysis of these fat co- and by-products led to propose a system of classification (Gasperini et al. *European Journal Lipid Science and Technology* 109, 673-681,2007).

A publishable “Handbook of analytical methods for fat control” has been also produced, compiling the complete procedures applied and recommended for the control parameters assessed in fats during the project.

1.4.2.Contamination levels in feed fats

According to the total PAH values (BF+BaP+BeP+Per+IP+DA+BP) detected, feed fats can be classified in three groups:

- Very low PAH content (<0.5 – 2 ng/g) : FISH, HYBY, LECI

- Very low PAH content, but potentially at risk (a reduced number of samples between 2 and 20 ng/g) : ANFA, EBE, FACS, MIX, RECY

- Low to very high content : AOCHE (max = 340 ng/g), AOPHY (max = 190 ng/g)

The results on dioxin (PCDDs/PCDFs) and DL-PCBs content indicated that the quality of feed fats was, in general, high. The values were mostly below the limits established by EC regulations (Commission Directive 2006/13/EC). Only 1 fish oil exceeded the maximum levels established for dioxins (6 ng WHO-TEQ/kg) and 2 fish oils exceeded the maximum levels established for dioxins+DL-PCBs (24 ng WHO-TEQ/kg). For the remaining fat categories, only 1 AOCHE exceeded the maximum level of dioxins (0.75 ng WHO-TEQ/kg for vegetal oils and by-products), and 2 HYBY and 5 AOPHY exceeded both the maximum level of dioxins and also that of dioxins+DL-PCBs (1.5 ng WHO-TEQ/kg for vegetal oils and by-products). The PBDE levels in the fat samples were negligible, always < 2 ng/g (there is no legal regulation on PBDEs in feeds and foods).

1.4.3.Relevance of the fatty acid composition of feed fats

We have reported before that the fatty acid composition of fat co- and by-products varies with the source. Thus, the by- and co-products of distinct fat categories are as follows: fish oils are very polyunsaturated; those derived from seed oils (LECI, AOCHE, AOPHY) moderately polyunsaturated; olive oil AOCHE and AOPHY very monounsaturated; animal fats, and tropical oils by-products quite saturated; and hydrogenated fats extremely saturated. Consequently, the respective use of these fats in feed formulations may alter the fatty acid composition of feed to differing degrees. Animals, as humans, have the capacity to modify their tissue fatty acid composition, to a certain extent, in response to dietary FA. Obviously, the effect is variable according to the animal species and to the body tissue. This project compared the effect of several fat co- and by-products included in poultry and rabbit feed on the final meat produced.

As an example, the Figure 1 shows the differences found in the FA compostion of chicken meats obtained from the 12 different treatments of the 4 trials. A good correlation was observed respect to the FA composition of the corresponding feeds. Thus, the 3 treatments of trial "Trans", including palm fatty acid distillate and its hydrogenated by-product, led to meats with the highest % saturated and *trans* fatty acid; the 3 treatments of trial PAH, incluidng acid oils from olive, led to meats with the highest % monounsaturated fatty acid; the 3 treatments of trial "Oxidation", incluiding oil blends rich in sunflower, led to meats with the highest % n-6 polyunsaturated fatty acid; and the treatments of trial "Dioxin", including fish oils, led to meats with the highest % n-3 polyunsaturated fatty acid.

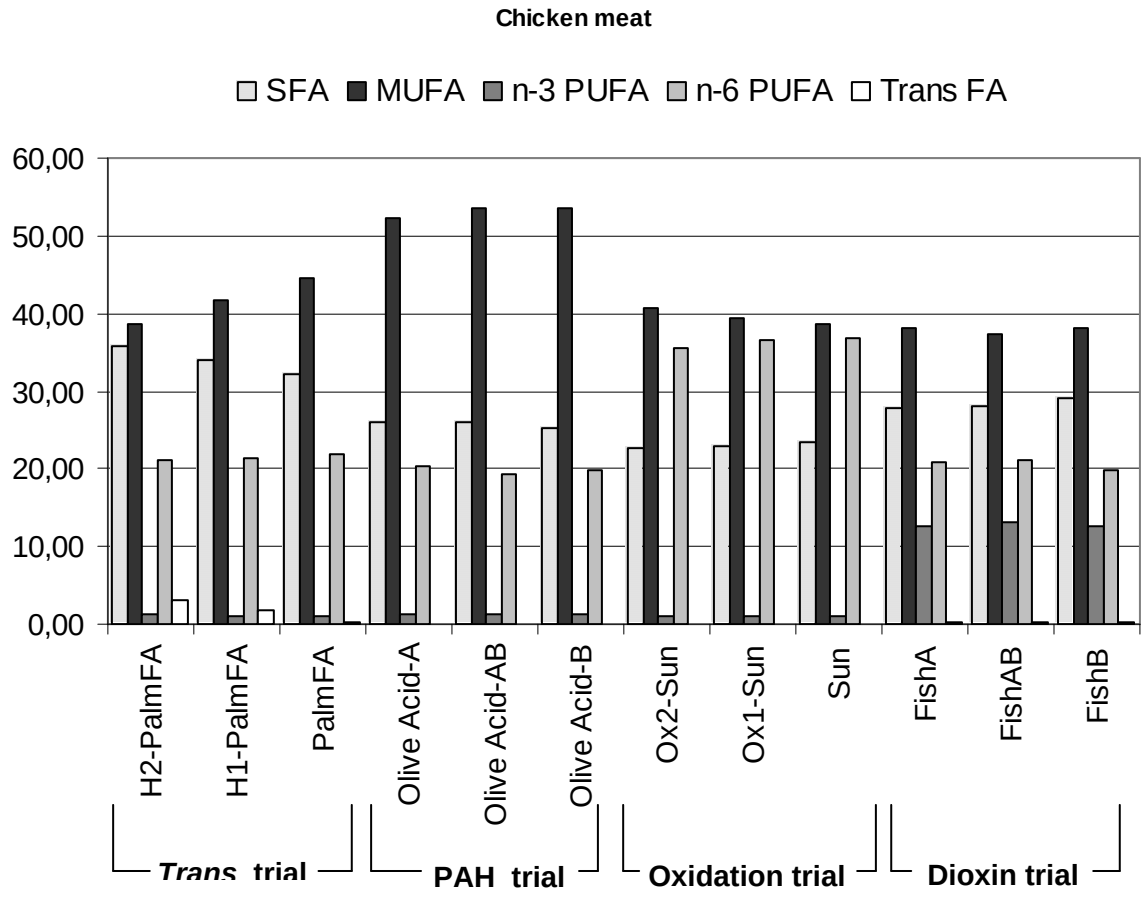


FIGURE 1-Fatty acid composition of chicken meats obtained from the 12 experimental feeds (H2 and H1= hydrogenated; Ox2 and Ox1= oxidated oils) (SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; Trans FA= total *trans* isomers of unsaturated fatty acids)

But the response in rabbit meat is something different. Although meat FA correspond quite well to the feed FA composition, saturated fatty acids were quite high in general, while in the case of chicken meat (Figure 1) we can observe that meats in general tend to be more monounsaturated. Regarding the liver FA composition, the behaviour is opposite to the meat. Thus chicken livers reflects worse the corresponding feed FA

composition, and the %SFA is the highest for all the treatments. In contrast, for rabbit livers the % of PUFA tend to be much more higher in all the treatments respect to chicken livers and to the meats.

Given that human “dietary reference intakes” have been established by international health organizations for several nutritionally essential FA (linoleic, linolenic, EPA, DHA, total n-3, and total n-6), the knowledge of the effects of feed fats can help meat producers to achieve a particular FA profile in meat and meat products. In addition to the nutritonal relevance of the fatty acid composition of meats, this composition is a key factor for the oxidative stability of meats. Meat lipid oxidation can give rise to off-flavors, which worsen when the food is cooked. As a rule of thumb, the more unsaturated the meat fat the less oxidative stability it shows. Finally, the level of unsaturation of meat and fat tissues affects texture properties. Thus, highly unsaturated feed can lead to oily carcasses or fat depots. In contrast, highly saturated feed may decrease the palatability of meat and meat products. In summary, balancing polyunsaturated FA with saturated ones would be optimum for meat quality, the former providing nutritionally valuable FA and the later suitable oxidative stability, texture and another sensory properties. However, lipid oxidation can be modulated by the presence of natural antioxidants, named tocopherols and tocotrienols, in meat and fat tissues. Thus, increasing polyunsaturation in meat would provide good results regarding meat quality, if tocopherol content were increased in parallel.

1.4.4.Transfer of tocopherols from feed fats to meat and liver, and relationship with oxidation levels and oxidative stability

Tocopherols and tocotrienols are the most active natural antioxidants in feed and food, and in tissues *in vivo*. These compounds are common components of oils and fats and, as commented earlier (see 1.4.1), several fat co- and by-products are particularly rich in these antioxidants. The concentration of tocopherols in meat and another animal tissues can be enhanced by increasing the concentration of these compounds in feed. Moreover, augmenting this concentration in a meat or fat tissue sample leads to a reduction in oxidative stability. Thus, the levels of tocopherols and tocotrienols in a feed fat can determine the oxidation level and the stability of meats or fat tissues obtained. Conclusions about the transfer of tocopherols/tocotrienols to chicken and rabbit meat are summarized as follows. In general, the transfer of tocopherols was higher for chicken meat (1.4 to 5.5 times) than for rabbit meat, although the chicken meat

included the skin and therefore its fat content was also higher (10,4% fat for chicken meat with skin, and 2,8% fat for rabbit meat). However, the influence of the total fat varied depending on the tissue. Thus, chicken and rabbit livers showed similar percentages of fat, but again contents of tocopherols were higher (1.5 to 4.3 times) for chicken liver than for rabbit liver. As an example, the Figure 2 shows the differences found in the tocopherol content in chicken meats and livers obtained from the 12 different treatments of the 4 trials. A good correlation was observed respect to the FA composition of the corresponding feeds. Finally, a relevant effect of the FA composition of the feed on the tocopherol transfer was found. Thus, the figure shows as usually tocopherol levels are higher in chicken meat than in the corresponding liver, except when monounsaturated FA are predominant in the feed FA composition.

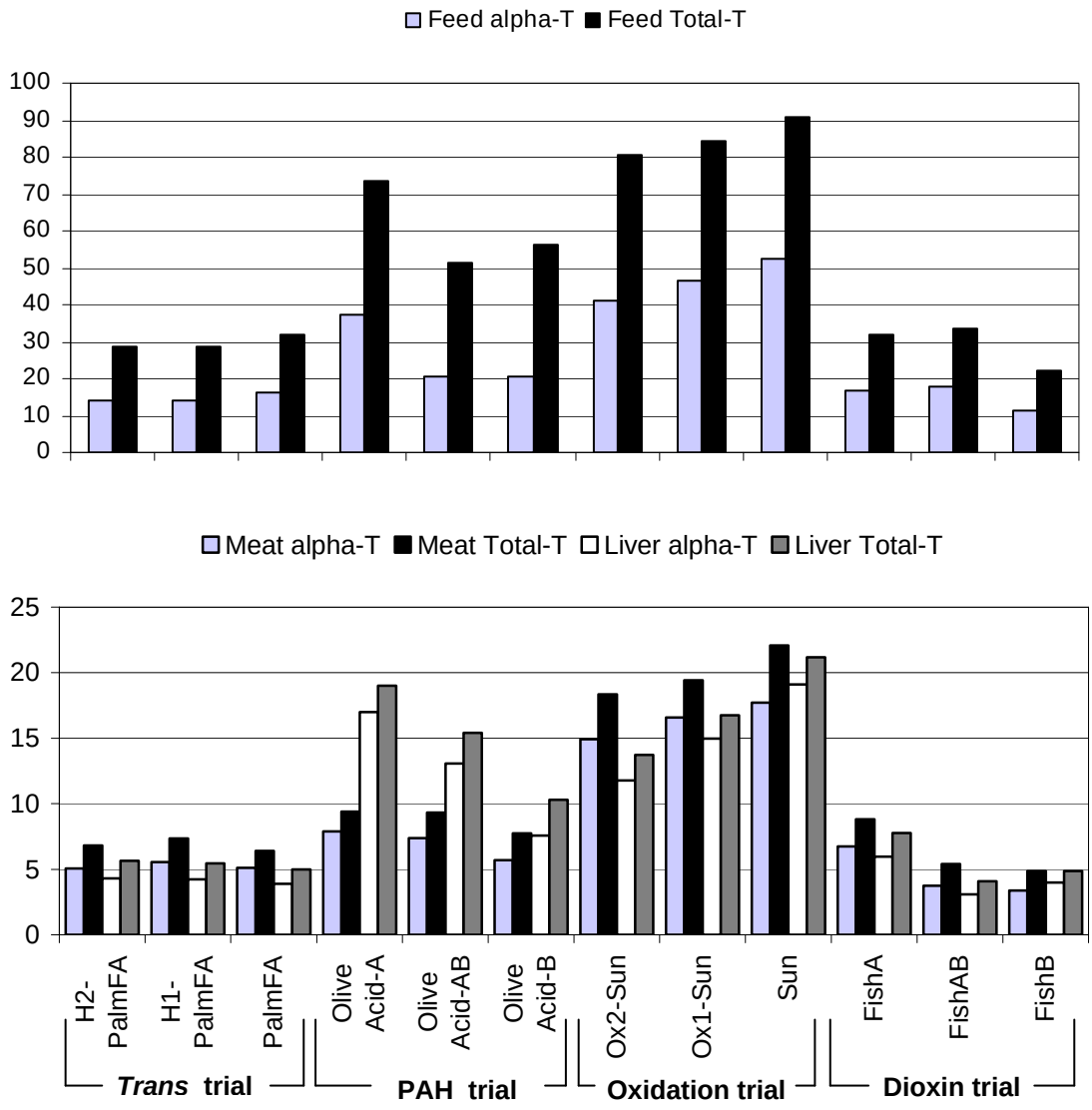


FIGURE 2-Tocopherol content in chicken meats and livers obtained from experimental feeds
(Alpha-T= alpha-tocopherol; Total-T= sum of tocopherols)

Regarding the levels of oxidation and the oxidative stability in meat and liver samples from these animal trials, only the use of fish oils (rich in highly polyunsaturated n-3 fatty acids) in feeds led to relatively high oxidation levels, measured as TBA value. When saturated, monounsaturated or n-6 polyunsaturated fats were included in feed, TBA values were very low. The same pattern was observed for the oxidative stability (measured as the induced Xilenol Orange colorimetric method). Feeds including fish oil caused meat and liver to be more susceptible to lipid oxidation. We tested in feed the addition of two fish oils with similar fatty acid composition but distinct tocopherol content, and the oil containing more tocopherols (Fish A) led to lower oxidation values in meat and liver of both chicken and rabbit.

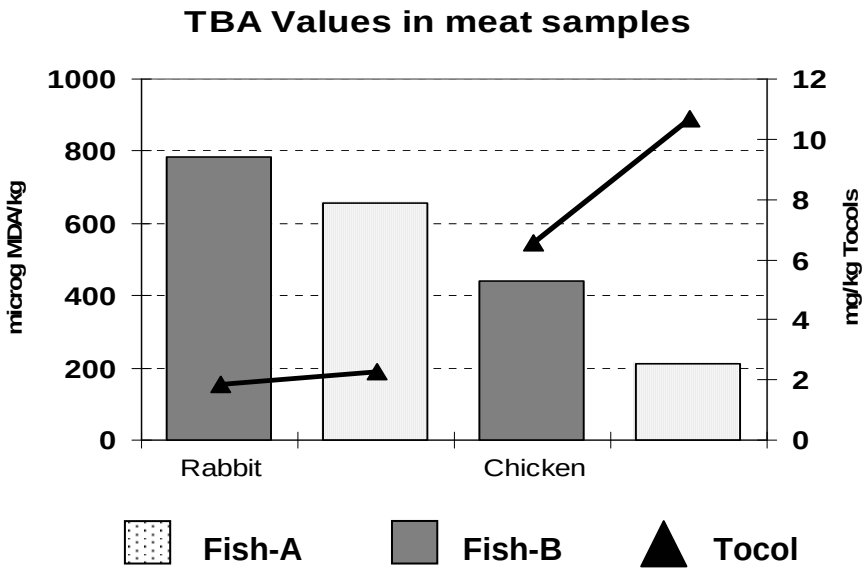


FIGURE 3-TBA values in rabbit and chicken meats and the corresponding total tocol contents
(Fish-A and Fish-B corresponded to both fish oils treatments; Tocol= sum of tocopherols+tocotrienols)

The Figure 3 showed the TBA values in chicken and rabbit meat corresponding to the treatments including two different fish oils (Low and High “Dioxin Trial” feeds). Bars indicate meat TBA values and triangles indicate tocopherol content in the same meat samples. An inverse correlation between both parameters can be observed. Moreover, rabbit meats show higher secondary oxidation values than chicken meats, and this fact is also explained by the much lower tocopherol contents observed in rabbit meats respect to chicken meats.

1.4.5.Transfer of trans fatty acids and CLA from feed fats to meat and liver

Previous studies have shown the capacity of TFAs to accumulate in muscle and fatty tissues. The results from this project showed that, both in chicken and rabbit meat, the TFA content was higher when hydrogenated fats were used in experimental feeds. When oxidated oils and fat refining by-products (AOCHE) were added to the feeds, low but significant content of TFAs in meat were detected. Some differences between species were observed. Thus, chicken meat with skin showed higher TFA content (2,6 to 5,4 times) than rabbit meat. But we have to take into account that chicken feed included 6% added fat and rabbit feed only 3%. Moreover, chicken meat includes the skin that is a fat deposit tissue, while rabbit meat only contain intramuscular fat. No CLA was detected neither in chicken nor in rabbit meat and liver, since the corresponding levels in feeds were not detectable.

1.4.6.Cholesterol and cholestrol oxidation products (COPs) in meat and liver

In both chicken and rabbit trials, dioxin feeds contained both cholesterol and phytosterol oxidation products (SOPs). The levels of SOPs were considerably higher in dioxin feeds compared with other feeds. This may be due to much higher oxidability of n-3 PUFA from fish oils in the dioxin feeds. The *trans* and PAH feeds also contained higher levels of SOP, compared with oxidized feeds used in chicken and rabbit trials.

Regarding chicken and rabbit meat, levels of cholesterol were very similar for all the experimental treatments, although some variations among the treatments were observed. Formation of COPs in meat was favoured when oxidated, *trans* or very polyunsaturated fats are added to the feed. In chicken and rabbit liver, cholesterol levels were higher than meat, but very similar profiles of cholesterol and COPs was found. The highest COP values were observed for liver coming from fish oil, *trans* and oxidized feeds, and the lowest from PAH feeds. This occurred may be due to domination of mono-unsaturated fatty acids in PAH feeds. The main quantitative difference, in general, was that cholesterol values were lower in rabbit liver, while COPs values were higher for rabbit livers respect to chicken livers.

1.4.7.Transfer of contaminants from feed to meat and liver

None of the PAHs was detected in meat or liver, even when extremely high total concentrations, from 1000 to 4000 ng/g, were present in the feeds. Hence, the transfer of PAHs from highly contaminated feed to animal tissues did not occur, confirming the high capacity of animals to rapidly metabolize these contaminants. In contrast, PAH metabolites were clearly visible in plasma, but only when very high contents of PAH were present in the feed. When contents corresponding to those usually found in fat by- and co-products were assayed, no PAH metabolites were found. However, metabolites are more toxic than the corresponding PAH. This indicates that, at very higher levels of PAHs in feed, it could exist some risk for animal health. However, taking into account the usual PAHs background contamination of feedstuffs, a health hazard to farm animals and to humans consuming animal products can be ruled out.

Dioxins (PCDD/Fs) and DL-PCBs show high stability and persist in the different environmental compartments for years. In addition, because of their liposoluble character and their low metabolization rate, in contrast with PAHs, they tend to accumulate in the tissues of living organisms. On the basis of the results obtained in the chicken and rabbit trials of this project, we conclude that these contaminants were transferred from feed to meat and liver. In general, the content of the individual dioxins and DL-PCBs in meat and liver usually increased when increasing concentrations are present in feeds. Our results show that dioxin contents and the sum of dioxin and DL-PCB contents in chicken meat samples coming from the feeds containing the highest values (a high contaminated fish oil feed) were above the maximum established at the European Regulation (COMMISSION REGULATION (EC) No 199/2006 of 3 February 2006). In contrast, when a feed containing a low contaminated fish oil was given to the chicken, the content of these contaminants in meat was lower than those maximum levels. The results of the “spike trial”, including increasing spiked concentrations of these contaminats in the feed fat, confirmed that a very good correlation exist between contents in feed fat and meat. The lower transfer observed for these contaminants from feeds to rabbit meat can be due to the use of half of the percentage of fish oil in rabbit feeds compared to the chicken feeds, and also to differences in bioaccumulation processes between the two types of animals. The presence of the skin in chicken meat samples is again a key factor in the highest capacity of dioxin and DL-PCB accumulation, respect to the rabbit meat.

Finally, we want to remark that the “Dioxin trial” was conducted using fish oils as feeding fats since they are the most naturally contaminated fats (regarding dioxins and PCBs). For this reason we have to raise two main conclussions. First, the use in feeds

of other fat categories that are usually less contaminated than fish oils, as we checked in the first part of the project, will lead to meats showing very low levels of these contaminants. Second, a good control of the fat contamination is required, since if we could assure low levels of dioxins and PCBs in feed fats we can be safe about the corresponding meat consumption.

1.4.8.Animal production implications

In order to evaluate the effects of feeding fat recycled materials, differing in their levels of oxidation or fatty acid isomers or PAHs or contaminants (PBDEs, dioxins and dioxin–like PCBs), on the animal performance, specifically on broiler chickens and rabbits, four feedings trials were performed in each species.

The feeding trials with broiler chickens were performed at the Experimental Farms of the University Autònoma of Barcelona (UAB) and the rabbit feeding trials were carried out at the University Politécnica of Valencia (UPV).

In both species, growth and digestibility trials were conducted. For each species, the same experimental protocols were followed in all the experiments. These protocols received prior approval from Animal Protocol Review Committee of both institutions. The protocols, housing, husbandry and slaughtering conditions conformed to current European Union guidelines.

The growing period lasted from 7 to 47 days of life for broiler chickens while the experimental period for rabbits lasted from weaning at 28-day old until 63-day old.

The use of hydrogenated palm fatty acid distillate with 12.4% of total *trans* fatty acids had some negative implications on growth performance and nutrient digestibility. But the fact that this fat source also contains a high percentage of saturated fatty acids does not allow us to conclude a net effect of *trans* fatty acid content on the impaired parameters. The use of fish oil with high content in dioxins and DL-PCBs (28.8 pg WHO-TEQ PCDD/Fs + DL-PCBs/g oil) did not cause negative effects on growth performance and digestibility, when compared with fish oil low in dioxins and DL-PCBs (9.64 pg WHO-TEQ PCDD/Fs + DL-PCBs/g oil), which originated some minor reduction in fat digestibility in broiler chickens and poorer growth performance in rabbits; consequently, these effects seem better due to the origin and to the quality of the fish oils than to their level of dioxins and DL-PCBs. The use of acid oils with different PAH levels (5290 ng or < 18 ng/g oil) did not cause differences on growth performance but the highest level originated some reduction in nutrient digestibility in both broiler chickens and rabbits.; These differences could be also due to the origin,

fatty acid profile and quality of the acid oils. The use of sunflower/olive oil before or after frying, with different levels of polymers (0.35% vs. 6.61%, respectively) and p-anisidine values (2.74 vs. 67.43, respectively), did not cause differences on growth performance or nutrient digestibility in both broiler chickens and rabbits.

1.4.9. Animal health implications

In order to asses the risks related to the use of recycled fats in the feed industry and animal production, regarding specifically broiler chicken and rabbit production, different diagnostic tools have been selected, as daily controls and records of incidences including deaths during the growing period; post-mortem inspection and the evaluation of different biochemical, microbiological or histological parameters.

Broiler Chickens

In all the trials, the inspection post-mortem made in collaboration with pathologist, did not show any remarkable organic lesion in the different organs studied.

In general, an improvement in the erythrocyte membrane integrity was showed as animal grew older. The haemolysis rate is an indicator of cell membrane integrity. No differences in the haemolysis rate were seen in the trials except for the TransFA study. Chicken fed a high TransFA diet showed after a week on trial, a higher haemolysis rate.

TBA values in blood samples**,** which represent the concentration of secondary oxidation products, were higher in the dioxin and PCB trial than in the rest. This effect cannot be related to the level of contaminants in fat because the highest values correspond to the low dioxin and PCB diet.

Gastrointestinal microbiota is a complex and dynamic ecosystem that inhabits the animal gut since birth and have an important influence on the host health: gut bacteria provide essential products to the host, form a key barrier again pathogens and also play important roles in gut morphology, immunity development, digestion and even modulating gene host expression.

The results have shown that the inclusion of different fatty recycled materials, differing in their levels of fatty acid isomers, dioxins and PCBs, PAHs or lipid oxidation, had not a major effect on the microbiota equilibrium, evaluated either by similarity analysis

(dendrogram representation) or as number of terminal fragments (biodiversity). The minor differences found in dendrogram analysis are in some cases inconsistent, appearing only in particular periods but not in the remaining. Even in some cases, as in the PAHs trial, changes would be associated to a faster establishment of the indigenous microbiota in the adult bird. Diversity of the ecosystem was neither different between diets in any period. However it has been clearly stated how the cecal microbiota changes along live with increases in their complexity that probably leads to a more stable community. Studying the microbial activity as the concentration of fermentative products, we could not detect any differences in the concentration of total SCFA or in the percentage of the different SCFA related to diets. However, lactic acid concentration varied between diets in the dioxin and PCB trial and in the lipid oxidation trial, increasing with the high level diets. Though other dietary effects cannot be discarded, these changes could reflect the effect of these contaminants and alterations on the microbial metabolism and/or on the microbial structure that would had not been detected by T-RFLP methodology. It is necessary to remind that PCR based fingerprinting techniques normally only take into account those microbial groups that represent more than 1% of total population disregarding other minor groups that could have an impact on the metabolism and in the fermentative activity of the global ecosystem. Regarding the effect of dietary fats on gut morphology, there were not differences between experimental treatments. Nevertheless, as age increases it is evident an increase in villi height and a proportional decrease in crypt depth and in the numbers of intraepithelial lymphocytes. It is important to state that even when statistical differences in the counts of *coccidia* in excreta were found, they never had a clinical implication.

Rabbits

An experiment was performed in order to test the possible effects of the factors under studying (levels of trans fatty acids, dioxins and PCBs, PAHs or lipid oxidation) in the used fatty recycled materials on the hepatic and renal functions, and on the caecal ambient.

The dietary level of *trans* fatty acids, dioxins and PCBs, PAHs or lipid oxidation seems not affect the hepatic function when evaluated by means of transaminase (GGT, GOT, GPT) and alkaline phosphatase blood levels. On the other hand, a relevant and statistically significant elevation of all transaminases was detected in rabbits feeding the low dioxin and PCB diet, suggesting a toxic chronic hepatitis induced by this diet

because of including some fish oil inappropriate for rabbits. Similarly, no effects of dietary treatments were observed on renal function, assessed by means of urea and creatinine blood levels. Finally, some effects of the dietary variations on caecal parameters were found, as lower total SCFA concentration with the high *trans* fatty acid diet, higher pH with the low dioxin and PCB diet and higher NH_3 concentration with the high PAH diet, all they being unfavourable for stability of caecal microbial ecosystem and intestinal health. Nevertheless, we cannot attribute them only to the mentioned factors because other ones can be involved in the compared fatty recycled materials.

In summary, under our experimental conditions and based on all the previously stated data, we can conclude that the inclusion in broiler chicken or rabbit feed of different fatty recycled materials differing in levels of trans fatty acids, dioxins and PCBs, PAHs or lipid oxidation had not major effect on animal health. Some of the differences found between treatments are more dependants on the origin, quality and profile of the dietary fat, than on the alteration itself.

1.5.Consumer opinion

The *Feeding Fats Safety* project included a study of European consumers' opinion on the use of fat co- and by-products in animal production and the effects of these products on the quality and safety of meat. This opinion can be relevant as a tool to evaluate the impact of the results obtained from the project. Results of the study can also serve to detect the main failures in cosumer information and to propose systems to improve it. Project researchers designed a questionnaire trying to cover the main aspects involved in the project in order to obtain the opinion of the consumer. The countries were selected trying to cover different lifestyles, cultural habits and population size. The characteristics of the study were as follows.

COUNTRIES: Spain, UK, France, Germany, Sweden, Poland (n=500 for each country). The total estimated population of these six countries corresponds to 60% of the total EU(25) population.
TARGET: General population (the distribution of the sample is national representative of each country in terms of sex, age, region and habitat)
QUESTIONNAIRE ADMINISTRATION METHOD: Omnibus telephone interviewing

The questionnaire included the following 10 questions.

1-Which of the following materials do you recycle at home?

- *Paper/cardboard*
- *Plastic*
- *Glass*
- *Organic matter*
- *Batteries*
- *None of them*

2-To what extent do you agree with the following affirmation: "Farm animal feeding influence on meat quality". Do you…

- *Strongly agree*
- *Somewhat agree*
- *Somewhat disagree*
- *Strongly disagree*
- *Don't know/Don't answer*

3-I am going to read you the description of a project that is being carried out in the European Union and I will ask you your opinion about.

"The project aims to establish the composition and quality of the fatty by-products of animal and plant origin. Some examples of these by-products are the fats extracted from butcher's and slaughterhouse's wastes or the fatty by-products obtained from olive or sunflower oil refining. Several of these fatty by-products are widely used to elaborate feeds for meat-producing animals. The avoidance of these fatty products in feed production would entail an environmental problem and an increase of meat price"

To which of the following affirmations do you agree more?

- *These fatty by-products should not be used to produce feeds.*
- *These fatty by-products should be exhaustively controlled before to be incorporated into the feeds.*
- *The use of these fatty by-products does not affect meat quality.*
- *Don't know/Don't answer*

4-What do you think about the idea of establishing the composition and quality of these fatty by-products in order to guarantee the meat quality and safety?

- *Very good*
- *Good*
- *Bad*
- *Very bad*
- *Don't know/Don't answer*

5-Do you think that the use of these fatty by-products in feed production involves a HEALTH RISK for the consumer?

- *Yes, due to the presence of toxic contaminants and micro-organisms*
- *Yes, due to the presence of toxic contaminants and their low nutritional value*
- *Yes, due to the presence of micro-organisms and their low nutritional value*
- *Yes, but I do not know why*
- *No, it does not involve any risk for the health*
- *Don't know/Don't answer*

6-To what extent do you agree with the following affirmation: "the use of these fatty by-products in feed production involves an ENVIRONMENTAL ADVANTAGE ". Do you…

- *Strongly agree*
- *Somewhat agree*
- *Somewhat disagree*
- *Strongly disagree*
- *Do not know/Do not answer*

7-To what extent would you be prepared to pay more for a meat coming from animals fed without these fatty by-products? Would you be…

- *Very prepared*
- *Somewhat prepared*
- *Not too prepared*
- *Not at all prepared*
- *Don't know/Don't answer*

8-To what extent do you think that is interesting that the European Community funds this study on fatty by-products? Would you say…

- *Very interesting*
- *Somewhat interesting*
- *Not very interesting*
- *Not at all interesting*
- *Don't know/Don't answer*

9-Which do you think that would be the main consequences of this study on fatty by-products for our society? (multiple answers allowed)

- *Better legal regulation on the utilization of these fatty by-products in feed production*
- *Better control systems for the utilization of these fatty by-products in feed production*
- *Increase of meat quality and safety*
- *Lower meat price*
- *More information for the consumer*
- *Don't know/Don't answer*

10-Who will take profit of this study on fatty by-products? (multiple answers allowed)

- *The fatty by-product producers*
- *The feed producers*
- *The farmer*
- *The consumers*
- *Don't know/Don't answer*

RESULTS OF THE CONSUMER OPINION

From the answers obtained after passing the questionnaire in the six countries, conclusions can be summarized as follows:

1. Paper/cardboard is the most recycled material (83%)
    a. Germany is the first country in recycling. On the contrary, Poland is the country with the least recycling habits.

2. The influence of farm-animal feeding in the quality of their meat is confirmed by the majority of the interviewees.
    a. Especially in Spain, where 79% of the population strongly agrees with this statement.
3. After knowing about the project that is being carried out on behalf of the European Community, 52% of our respondents agree with the statement “The use of these fatty by-products in cattle feeds is convenient, but this should be subject to exhaustive quality control”.
    a. Only 6% consider that the fatty by-products do not affect the quality of the meat.
4. The majority of the interviewees (84%) thinks that it is positive to measure the composition and quality of the fatty by-products in order to guarantee the quality and safety of the meat we consume.
5. There is a general perception about the existence of health risk for the consumers when using fatty by-products in feed production. Although, one third of the respondents do not know why.
    a. This perception is especially noticeable in Spain (50%).
    b. On the other hand, respondents from United Kingdom mention various reasons why it entails health risk. 46% affirm there is a presence of toxic contaminants and low nutritional value.
6. Nearly half of our total sample disagrees *somewhat* or *strongly* with the statement “The use of these fatty by-products in feed production is environmentally friendly”, (mean value=2.3, on a scale from 1 to 4, where 1 means “strongly disagree” and 4 means “strongly agree”).
7. There is a positive predisposition to pay more for meat from animals fed without the fatty by-products (77%).
8. The majority of the interviewees consider interesting the fact that the European Community is financing this study (74%).
9. “Better meat quality and safety” (59%), “better control systems for the utilization of fatty by-products in feed production” (58%) and “more information for the consumer” (57%) are the main consequences expected by the interviewees that this study will have on our society.

10. A majority (61%) of the respondents thinks that the consumer will benefit from this study on fatty by-products.

1.6.Final remarks

From our results, and from previous information, we can make some final remarks:

- They exist some fat materials obtained as co- and by-products of the food chain that can provide valuable characteristics in feeding animals. The most relevant question is to ensure a suitable level of quality and safety of meat and another foods from animal origin.
- Consequently, a higher level of standardization should be promoted, particularly for some of these feed fats. We checked that they exist enough analytical parameters to characterize and control their quality and safety levels.
- For some feed fat categories adequate system of purification should be studied in order to increase quality and safety of their use. Other fats need only a better system of traceability control and good manufacturing practices to reach suitable quality levels.
- Polycyclic aromatic hidrocarbons (PAHs) are contaminants that could be found in high amounts in some feed fats (acid oils), but they do not accumulate in animal tissues and have any repercussion on safety of foods from animal origin. In any case, some recommendations can be given in order to avoid these contaminants during some oil processing steps or to eliminate them by means of a subsequent special processing step. To prevent their formation it is necessary to dry seeds and nuts avoiding contact with exhaust. If PAH's are present, both in oils or co-products, they can be completely removed through filtration with 1 – 3% of active carbons. The extra-costs for the supplementary filtration with active carbons is negligible and acceptable from economical point of view"
- In contrast, dioxins and DL-PCBs are highly accumulated in animal tissues because of their liposolubility. Some feed fats, particularly fish oils, can show high levels of these contaminants. Our results show that is easy to exceed maximum levels regulated by the EU, particularly in the most fatty tissues, when moderately

contaminated oils are used as feedstuff. This fact pose a question about the need of new studies establishing relationships between the regulated feed maximum levels and the regulated meat and another foods maximum levels of dioxins and DL-PCBs.

- Concerning dioxin and PCB levels, which resulted to be higher than maximum admitted levels in some fish oils samples, no technological recommendations can be given. The contamination is of environmental origin and there are no cleaning methods at the moment for oils contaminated with dioxin and PCB which are economically acceptable. A method to improve fish oil safety could be the analysis of the fish oil stocks. Moreover, the oxidation control in fish oils is more problematic than for other co-products. To reduce or eliminate oxidation from fish oil, a refining treatment can not be applied, since significantly would increase the cost of this co-product in comparison with crude conventional oils.
- From the comparison of costs of iso-energetic feed formulas containing co-products or conventional oils, we can conclude that the use of co-products such as vegetable AOCHE, AOPHY and ANFA are economically interesting alternatives for feed and meat producers if compared with conventional oils such as soybean, rape, sunflower and palm oil.
- Considering that not only dietary energetic sources such as conventional oils and fats, but also cereals are used in increasing amounts for bio-fuels production, causing increasing oils prices, it makes sense to spend efforts to optimise the use of safe fatty co-products as dietary energy sources in animal nutrition.
- From our analysis resulted that the advantages of the use of some co-products and technical lipids in feed are independent from environmental benefits. Their use has reasons in itself and not as an alternative use to disposal

After the conclusion of FFS project the knowledge level on the composition and on the properties of feeding fats was greatly improved and one of the main goals we achieved was that is was possible to idenfy risk and benefits for the use of each fat category in feed formulation. This fact, along with a strong classification system and with the related available analytical protocols will ensure a better product evaluation, providing stronger rules for the food safety and for a fair trade.

2.DISSEMINATION AND USE

2.1.Dissemination activities during the project

2.1.1 Website of the project

The responsible for the coordination of activities related to the website of the project has been Dr. Codony (coordinator of the project). The design of pages and the distribution of the type of contents inside each webpage was agreed by all the partners. The website of the project was considered as the most efficient tool to disseminate our activities and to give periodic information of our results. For tese reason we decided that the website was active as soon as possible. The website of the project (http://www.ub.edu/feedfat/) is active since October 2005, and it is constituted by the following pages:

- "Home" page, including a summary of the project and information about the objectives, structure, and potential impact of the results. It also include news and events' information related to food and fedd safety
- "Participants" page, including information about each partner, the corresponding institution and contact data
- "Workplan" page, including more detailed information about objectives, structure, and work plan of each work package.
- "Documents" page, including two type of information. First, a list of presentations, articles or other products coming from the project. Second, reference documents and legal regulations related with the subject of the project. In most cases, the corresponding link to the document is available.
- "Meetings" page, including information about the meetings of the consortium.
- "Links" page, including links with another websites of interest in the field of food and fat quality and safety, corresponding to another EU project, EU institutions, Safety agencies, etc

The coordinator, Dr. Codony, has been the responsible for the management and updating of the contents. The rest of the partners have collaborated in sending suggestions and information to the coordinator, in order to update and improve the website. The website will be active at least during the following 2 years after the end of the project, since the activities of dissemination will continue in the next future.

2.1.2 Elaboration of scientific articles and presentation of communications

Publications

-M. Ábalos, E. Abad, J. Parera, M.G. Martrat, J. Sauló, J. Rivera. *PCDD/Fs and DL-PCBs in meat samples from chickens and rabbits fed with fish oil spiked feed at different levels of contamination*. Organohalogen Compounds 69, 106-109 (2007)

-M. Ábalos, J. Parera, E. Abad, J. Rivera. *PCDD/Fs and DL-PCBs in feeding fats obtained as co-products or by-products derived from the food chain.* Chemosphere 71, 1115-1126 (2008)
-Choque-López J.A., Baucells M.D., Mateus E.F., Gómez de Segura A., Barroeta A.C. *"Efecto de la alimentación con materia grasas recicladas sobre parámetros productivos y el rendimiento a la canal de pollos de carne"* XII Jornadas sobre producción animal. Comunicación. En ITEA, Vol. Extra Nº 28. Tomo I, 210-212 (2007)
-Gómez de Segura A., Castillo M., Takahashi S.E., Choque-López J.A., Barroeta A.C., Baucells M.D., Martín-Orúe S.M. *"Terminal restriction fragment length polymorphism (T-RFLP): Una herramienta útil para valorar la dinámica y la diversidad de la población microbiana en aves y cerdos"* Avances Metodológicos en el Estudio de la Microbiología Digestiva. Facultad de Veterinaria de la Universidad de Zaragoza. Pag 24-25. Zaragoza. (2007)
-G. Gasperini, E. Fusari, L. Della Bella, P. Bondioli. *The classification of fats for feeding purposes: a proposal*", Eur. J. Lipid Sci. Technol. 109, 7, 673-681(2007).
-Choque-López J.A., Manzanilla E.G., Gomez de Segura A., Baucells M.D. and Barroeta A.C. "*Effects of oxidation and vitamin E inclusion on resistance to haemolysis, intestinal microbiota, faecal coccidia counts and epithelium structure of broiler chicken"* in Avian Gut Function in Health and Disease. Poultry Science Symposium Series. Vol. 28 Edited by G.C. Perry. CABI. UK pag. 384. (2006)
-M. Ábalos, E. Abad, J. Parera, J. Sauló, M.G. Martrat, E. Rodríguez, J. Rivera. *Evaluation of PCDD/F and DL-PCB presence in feeding fats obtained as co-products or by-products derived from the food chain.* Organohalogen Compounds 68, 1862-1865 (2006)

Future publications

- University of Barcelona team are preparing the following articles:

-Fatty acid composition of chicken and rabbit meat, liver and plasma as affected by using fat co- and by-products in feeds
-Transfer of tocols from feed to meat, liver and plasma, in chickens and rabbits fed fat co- and by-products
-Oxidation levels and oxidability in chicken and rabbit meat, liver and plasma fed fat by-products

- University Autonomous of Barcelona team are preparing the following articles:

-Dynamics of Microbial Caecal Lumen Ecosystem and Histomorphology Development of Broiler Chickens with Age
-Effects of Dioxins and PAHs in Feeding Fats from the food chain on Productive Parameters, Gut health and Caecal Microbiota of Broilers Chickens.
-Effects of altered feeding fats (Oxidized and Trans fatty acids) on Caecal Microbiota and Productive Parameters of Broilers.
-Effects of the inclusion of different levels of dioxins and PCBs on Productive Performances and Faecal Microbiota

Journals to which the foreseen publications were sent:
_ FEMS Microbiology Ecology
_ Veterinary Microbiology
_ Poultry Science

- University of Bordeaux1-CNRS team are preparing the following articles:

-Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in various feeding fats materials obtained as co- or by-products from the food chain and used in animal production.
-PAH and PAH metabolites contents in tissues of broiler chickens and rabbits fed i) with experimental feed containing acid oils from chemical refining, and ii) with spiked experimental feed.
-Transfer rate of PBDE from low level spiked feed to meat and liver of broiler chickens.

- University Politechnic of Valencia team are preparing the following articles:

-Use of palm fatty acid distillates for feeding fattening rabbits: effects on nutrient digestibility, performance and health. Sent to World Rabbit Science.

-Effects of acid oil quality on nutrient digestibility, performance and health of fattening rabbits. Sent to World Rabbit Science.

-Use of highly oxidized vegetable oils in fattening rabbits: effects on nutrient digestibility, performance and health. Animal Feed Science and Technology.

-Effects of fish oil quality on nutrient digestibility, performance, carcass colour and health of fattening rabbits. Animal Feed Science and Technology.

- CSIC-Barcelona team are preparing the following articles:

-A publication related to the *"Effects of feeds containing fish oil with different level of contamination on the dioxin and DL-PCB contents in chicken and rabbit meat"*

-A publication related to the *"Rate of transfer of dioxins and DL-PCBs from feeds to chicken meat and liver"*

- SILO s.r.l. team are preparing the following articles:

-Socio-economic benefits and risks of the use of fat by- and co-products in animal feeding". Accepted to be published in two parts by the Rivista Italiana delle Sostanze Grasse, are being prepared by M. Parini.

- Stazione Sperimentale Oli e Grassi team are preparing the following articles:

-Handbook of analytical methods for feeding fats evaluation. A first alternative is to find and editor for the preparation of a printed volume. In absence of better solution we shall support the idea to make it available for downloading on the FFS website.

- Swedish University of Agricultural Siences team are preparing the following articles:

-Distribution of sterols and sterol oxidation status in feeds fats and oils used in Europe obtained from chemical and physical refining processes. Ubhayasekera S.J.K.A., Dutta P. C. (2008).

-Sterols and sterol oxidation products in feeding fats and oils used in Europe obtained from various by-product sources. Ubhayasekera, S. J.K.A., Dutta, P.C. (2008).

-Effects of feeds containing dioxins, PAH, trans fatty acids and oxidized lipids on cholesterol and cholesterol oxidation in chicken tissues Ubhayasekera, et al. (2008).

-Effects of feeds containing dioxins, PAH, trans fatty acids and oxidized lipids on cholesterol and cholesterol oxidation in rabbit tissues Ubhayasekera, et al. (2008).

Oral and poster communications presented

-Blas E., Ródenas L., Martínez E., Pascual J.J., Cervera C. *Effect of dietary fat quality on performance and health of fattening rabbits*. 8th World Rabbit Congress, Verona (Italy), (2008).

- Choque-López, J.A., A. Gómez de Segura, S.M. Martín-Orúe, S.E. Takahashi, A. Schiavone, A.C. Barroeta, M.D. Baucells. *"Dioxins and PCBs, and PAHs in Feeding Fats on Caecal Microbiota of Broilers Evaluated by Terminal Restriction Fragment Length polymorphism (t-RFLP)*". XXIII World's Poultry Congress Brisbane, Australia. 30 June-4 July (2008)

- Schiavone, A., J.A. Choque-López, M.D. Baucells, A.C. Barroeta. "*Oxidative Status of Dietary Fat Influences Lipid Stability of Erythrocytes in Chickens"*. XXIII World's Poultry Congress Brisbane, Australia. 30 June-4 July (2008)

- Gómez de Segura, A., Takahashi, S.E., Choque-López, J.A., Martín-Orúe, S.M., Sala, R., Barroeta, A.C., Baucells. M.D. "*Dynamics of Microbial Caecal Lumen Ecosystem of Broiler Chickens with Age Measured by Terminal Restriction Fragment Length Polymorphism (t-RFLP)"*. Gut Microbiome, Clermont Ferrant, France (2008)

- Choque-López, J.A., M.D. Baucells, E.F. Mateus, A. Gómez de Segura, A.C. Barroeta. **"***Effects of feeding recycled fatty materials on broiler chickens performance and carcass yield".* XVIII European Symposium on the Quality of Poultry Meat and XII European Symposium on the Quality of Eggs and Egg Products of WPSA. 2-5 Septiembre, Praga-(Czech Republic); 2-5 September (2007)

-M. Ábalos, E. Abad, M.G. Martrat, J. Parera, J. Rivera. *Levels of dioxins, furans and dioxin-like PCBs in meat of broilers fed experimental feed containing different amounts of these contaminants.* XVIII European Symposium on the quality of poultry meat & XII European Symposium on the quality of eggs and egg products. Praga (Czech Republic); 2-5 September 2007. Poster. Symposium Proceedings, p. 193 (P-001) (2007)

-Choque-López J.A., Baucells M.D., Mateus E.F., Gómez de Segura A., Barroeta A.C. *"Efecto de la alimentación con materia grasas recicladas sobre parámetros productivos y el rendimiento a la canal de pollos de carne"* XII Jornadas sobre producción animal. Comunicación (2007)

-Navarrete C., Martínez E., Ródenas L., Moya V.J., Pascual JJ., Blas E., Cervera C. *The use of palm fatty acid distillate and fish oil in fattening rabbit diets.* XXXII Symposium de Cunicultura - II Congreso Ibérico de Cunicultura de Asociación Española de Cunicultura (ASESCU), Vila-Real (Portugal), (2007).

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-Ubhayasekera, S. J. K. A. and Dutta, P. C. (2008) High levels of sterols and sterol oxidation products in some feeding fats obtained from co- and by-products from the food chain in Europe. 99th AOCS annual meeting & expo, 18-21 May, 2008, Seattle, Washington, USA. Oral presentation

-A conference communication has been asked for at the 2nd EuCheMS Chemistry Congress, September 16-20, 2008, Torino, Italy (deadline for summary submission: 25 April). If the conference is accepted, the whole results obtained in the Feeding Fats Safety program by the team of the University Bordeaux 1 will be presented.

-A poster presentation is going to be prepare on the last results of the WP4 for the 28th International Symposium on Halogenated Persistent Organic Pollutants. Dioxin'2008, Birmingham (UK); 17-22 August 2008

2.1.3 Organization of Specific Workshops or Conferences

The consortium organizes two Workshops along the duration of the project, with the aim to present the most relevant results to experts and scientists, as well to consumer and professional associations representants. We invited also to all the fat factories that have collaborated with the project in sending fat samples. These workshops were a good oportunity to discuss our results from an external point of view and reinforce the conclusions of the project. The workshops organized were two:

- *One-Year workshop* (Barcelona, January 2006)

As a complementary session of our One-Year Meeting the consortium programmed this workshop including a series of presentations to provide information and stimulate discussion on the results of the characterisation of feeding fats, analysed during WP1 (first year of the project). As not all the fat suppliers and observers could attend our meeting, after the meeting we sent out to everyone the pdf files of all these presentations, summarised below:

- WP1 management and fat sample collection and distribution
- Results of Chemical Composition of fat feedstocks
- Results of Degradation compounds in fat feedstocks
- Results of Sterol oxidation products contents
- PHA & PBDE contamination of fat feedstocks
- Dioxins and PCB contamination of fat feedstocks
- Proposal of a system for the classification of fat feedstocks

- *Three-Year workshop* (Barcelona, March 2008)

As a complementary session of our Final Meeting the consortium programmed this workshop including a series of presentations to provide information and stimulate discussion on our results about the effects of fat co-products on meat and animals. These presentations were the following:

- Chemical characterisation and classification of fats
- Repercussions on animal performance and health
- Repercussions on meat lipid quality

- Socio-economic benefits and risks of the use of fat by- and co-products in animal feeding
- PCCD/PCCF and DL-PCB in fats,rate of transfer to animal tissues and repercussions on meat safety
- PAH in fats, rate of transfer to animal tissues and repercussions on meat safety
- European consumer opinion
- Main reports and publications coming out from the project

All the presentations are available at the website of the project.

2.1.4.Specific reports for consumers, and productive sectors

These reports are designed to cover the 3 main areas of the project, in order to spread the knowledge and improve their use inside a safety and quality approach of meat production. They must be focused on results of the project and previous knowledge, emphasizing the advantages and drawbacks of the use of these recycled fat materials and the applicability of these feeding fats in animal/meat production with high levels of quality and safety. This three reports are,

1. **Report** on the main applications and advantages of the use of these recycled fat materials will be prepared for communication to **fat and feed producers** (coord. Dr. Bondioli, SSOG).

2. **Report** on the applicability of these feeding fats in meat production will be prepared, to spreading the knowledge and improved the use of these fats between **meat producers** (coord. Dr. Blas, UPV).

3. **Report** on the safey and quality characteristics of a meat production system based on the use of recycled fat materials, adressed to the **consumers** (coord. Dr. Codony, UB).

This three Reports are designed to be directly publishable. At this moment the consortium is still studying the best way of publication, in order to have the widest level of dissemination. Professional and consumer European associactions and federations are being contacted asking for a possible interest in the publication.

2.2.Final plan for the use and exploitation of knowledge

2.2.1.Periodic dissemination activities of information to the productive sectors and the consumer

- Brochures and dissemination articles:

  *Rivista Italiana delle Sostanze Grasse (vol LXXXII, Jan-Feb 2005): "Il progetto di recerca europeo *FEEDING FATS SAFETY*"

  *Bulletin CESFAC-Info General 08 (24 March 2006): "Presentación del primer año del proyecto *Feeding Fats Safety*"

  *EUROCARNE (Nº 163, Jan-Feb 2008): "Caracterización de la calidad y seguridad de subproductos grasos de la cadena alimentaria para su uso en piensos. Proyecto europeo *Feeding fats safety"*.

  *In SILO brochures on company activities FFS project is always included, giving relevant web-site coordinates.

- 26th World Congress and Exhibition of the ISF (Praga, September 2005): Poster presentation, " *Feeding Fats Safety* Project. Improving the use of feeding fats materials coming from by- or co-products from the food chain: a quality and safety approach".

- EFPRA Meeting (Marbella, June 2007): Oral presentation made by Dr. Codony (coordinator), *"*Quality and safety of feeding fats obtained from co-products or by-products from the food chain*"*

- XVIII Eur. Symp. Quality of Poultry Meat of WPSA (September 2007): Oral presentation by A.C. Barroeta (UAB), "Use of Fat Co- and By-products in Poultry Nutrition"

- FEEDINFO electronic publications:

  *Using Fat Co- and By-Products from the Food Chain as Dietary Energy Sources in Animal Nutrition Makes Sense, Says European Team of Experts (April 2008)

  *Socio-economic impact of the application of recycled fatty materials in commercial feed (April 2008)

- CESFAC Technical meeting (Madrid, 23 May 2008): the coordinator, Dr. Codony, has been asked for a presentation of main findings and conclusions of the project at the technical meeting of CESFAC (Spanish associate to FEFAC).

For the distribution of the results of fat co- and by-products characterization among the factories collaborating in supplying samples, Dr. Bondioli and Dr. Parini prepared two files, one for the "Chemical properties" and another for "Contaminants". These files

contained all the results corresponding to the analyses perfomed, keeping the obliged confidentiallity by assigning aleatory codes to the samples.

2.2.2.Contacts and collaboration with another researchs groups and EU Framework Programme projects

-An agreement of collaboration was signed with the CRA-Wallonie (Belgium) on fat samples analysis. Dr. Vincent Baeten asked for the possibility to analyse some of our fat samples collected during WP1:

- They are interested on the detection and differentiation of animal fats according to the species
- Analytical expertise on Raman spectroscopy and chemometrics
- A first communication in common will be presented: Abbas O., Codony R., von Holst C., Baeten V. *Assessment of the discrimination of animal fat by FT-Raman spectroscopy*, lecture that will be given by Dr. Baeten at the XXIX European Congress on Molecular Spectroscopy, Opatija, October 2008.

-Feed Safety Platform participation: Feeding Fats Safety Project was invited to participate in this platform that includes other EU Framework Programme projects. Moreover, this connection can be interesting for:

- A higher level of integration of the members of the FFS consortiumin into a bigger platform for the development of the research in the field of food and feed safety
- The possibility to participate in some future European projects and another activities (7FP or another European programmes)

-Feed Safety Conference, Namur, November 2007. The coordinator Dr. Codony was invited to the Conference organized by the CRA-Wallonie:

- The Conference was of high interest, because of the specificity on Feed Safety, and for the high level of the participants
- Interesting to generate new contacts and to reinforce the FFS consortium for future actions and for spreading results
- Presentation during this Conference of a poster containing the most relevant results of the FFS project: *"FEEDING FATS SAFETY" Project. Characterisation of feeding fats coming from by- or co-products from the food chain. Quality and safety for meat production.*

-An agreement of collaboration was signed with RIKILT-Wageningen (The Netherlands). Dr. Saskia van Ruth asked for the possibility to analyse our fat samples collected during WP1, in order to apply new techniques for completing the characterization of our fat samples or to get new proposals for classification:

- They are interested on the detection of ruminant fats and also in the differentiation of animal and vegetal origin.
- Analytical expertise on Proton Transfer Reaction Mass Spectrometry and chemometrics (PCA) applied to major and minor composition

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  - Paolo Bondioli (Stazione Sperimentale Oli e Grasssi, Milan)
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  - Maria T. Rodríguez Estrada (university of Bologna)
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  - Esteban Abad (Laboratory of dioxins-CSIC, Barcelona)
  - M. Dolores Baucells (University Autònoma of Barcelona)
  - Enrique Blas (University Politècnica de Valencia)
  - Manuela Parini (SILO s.r.l., Florence)

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