PCDD/Fs AND DL-PCBs IN MEAT SAMPLES FROM CHICKENS AND RABBITS FED WITH FISH OIL SPIKED FEED AT DIFFERENT LEVELS OF CONTAMINATION

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Abstract

A study has been carried out in the framework of the FEEDING FATS SAFETY project (6th EC Framework Programme) with the aim to evaluate the presence of PCDD/Fs and DL-PCBs in meat samples from animals fed with fish oil spiked feeds. Two fish oils, namely A (low contaminated) and B (high contaminated), were selected as an example of a wide variety of fats (by-products or co-products obtained from the food chain) that can be used as ingredients for feeds. Separate groups of two animal species, chickens and rabbits, were fed three different experimental feeds prepared by adding a certain percentage of one or both fish oils to a vegetal mixture base. Levels and congener distribution profiles of PCDD/Fs and DL-PCBs in the fish oils, the feeds and in the chicken and rabbit meat samples from the three separate treatments are reported. Significant differencies have been found in PCDD/F bioaccumulation in meat between the two animal species considered.

Introduction

Nowadays, there is a great concern in controlling different aspects of animal nutrition in order to preserve animal health and to produce safe and good quality meat products at the same time. The main objective of the FEEDING FATS SAFETY project, included in the 6th EC Framework Programme, is related to these topics. A specific work package of this project deals with the effects on animal production derived from the use of selected fats (by-products or co-products obtained from the food chain) as ingredients of the feeds. In particular, one of the parameters to be examined is the final levels of polychlorodibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and "dioxin-like" polychlorinated biphenyls (DL-PCBs) in the meat.

PCDD/F and coplanar PCB levels in muscle, liver and other tissues from chickens that were fed a experimental feed spiked with a mineral oil have already been determined[1]. Another work, also described the analysis of PCDD/Fs and PCBs in fat samples from pigs and broilers fed with contaminated feed obtained from a dilution of chicken feed from the Belgian dioxin crisis (1999)[2]. Recently, an episode of dioxin contamination dealing with a recycled fat from animal origin reused as an ingredient for the production of pig feed[3], demonstrates the need for intensive monitoring programs on these kind of by-products that are already being used as feed ingredients or can be potentially considered to this purpose.

In this study, separate groups of two different types of animals, chickens and rabbits, were fed with three different experimental feeds containing certain percentages of two selected fish oils. Levels of PCDD/Fs and DL-PCBs were determined in meat samples after a rearing period similar to that usually needed in animal production. Fish oils were selected instead of other type of fats since, in a previous study[4], they showed the highest levels for these contaminants among a large group of different categories of fat by-products or co-products from animal or vegetal origin.

Materials and Methods

Two fish oils collected in the framework of the FEEDING FATS SAFETY project were selected and classified as: Fish Oil A (low level of contaminants) and Fish Oil B (high level of contaminants). Fish Oil B was spiked with known amounts of EPA-1613PAR and WP-STK (Wellington Laboratories Inc., Guelph, Canada), which

contain native PCDD/Fs and DL-PCBs, respectively, in order to raise the levels of these contaminants above the maximum established at the EU Directive[5] for this kind of matrix.

Three feeds for each type of animal (chickens and rabbits) were prepared by adding different percentages of the fish oils to a mixture base mostly composed of raw materials of vegetal origin. The aim was to obtain different levels of PCDD/F and DL-PCB contamination in the final feeds, then three separate treatments could be carried out on the two species of animals. Chicken feed for Treatment 1 contained 6% of Fish Oil A; whereas, a mixture of 3% Fish Oil A and 3% Fish Oil B was used to prepare chicken feed for Treatment 2 and, finally, 6% Fish Oil B was added to obtain chicken feed for Treatment 3. In the case of rabbits, the three feeds were prepared in a similar way but using half of the percentage of fish oil added to the chicken feeds.

Groups of 4 broilers (5 days old) or 5 rabbits (28 days old) were fed for 42 days and 35 days, respectively, with one of the above mentioned feeds. Each one of these separate groups of 4-5 animals constitutes a replicate. In total, six replicates were considered for each of the treatments and animal species. At the end of the experimental period, the animals were slaughtered and the meat from their legs was taken and ground in order to have homogeneous samples.

Meat samples were then sent to the Dioxin Laboratory of the IIQAB-CSIC for PCDD/F and DL-PCB determination. Samples were freeze-dried and re-homogenized again prior to the analysis. Next, samples were spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS, Wellington Laboratories Inc., Guelph, Canada) and $^{13}C_{12}$-DL-PCBs (WP-LCS, Wellington Laboratories Inc., Guelph, Canada) and then extracted in a Soxhlet for ~24h with toluene:cyclohexane (1:1). The extracts were rotary evaporated and kept in the oven overnight (105 ºC) in order to eliminate the solvents prior to gravimetrical fat determination. Afterwards, fat residues were dissolved again in *n*-hexane. Organic components, fat and other interfering substances were removed by treating the *n*-hexane extracts with sulphuric acid. The extracts were then rotary concentrated and filtered prior to the next clean-up step. Further sample purification and instrumental analysis by high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) are described elsewhere[4].

Results and Discussion

Levels of PCDD/Fs and DL-PCBs were determined in the two fish oils, the raw materials and the experimental feeds at the beginning of the study. Fish Oil B was spiked with additional amounts of these contaminants in order to obtain a fat ingredient for feed with PCDD/F and DL-PCB levels not very similar to those originally present in Fish Oil A and above the maximum allowed in the EU Directive[5]. After being spiked, Fish Oil B showed significant higher concentrations (i.e. from 2 to 18 times higher) for most of PCDD/F and DL-PCB individual congeners compared to Fish Oil A. Major contribution to the PCDD/F Fish Oil B profile was from 2,3,7,8-TCDF (19%) followed by 2,3,4,7,8-PeCDF and OCDD with a percentage contribution of 10% each. On the other hand, Fish Oil A was characterized by a very large concentration of OCDD, even higher than the level obtained in the spiked Fish Oil B, OCDD represented a relative percentage of 47% of the total PCDD/F concentration, expressed in pg/g Fish Oil A. In general, the raw materials of vegetal origin, had no detectable or very low levels of PCDD/Fs and DL-PCBs compared to those found in the fish oils. For this reason, the amounts of these contaminants observed in the feed samples resembled in a large extend the theoretical expected levels that were calculated taking into account only the contribution of the percentage and the type of fish oil (A and/or B) added to obtain the corresponding feeds. Exceptionally, the determined levels of 1,2,3,4,6,7,8-HpCDD and, particularly, OCDD, were higher than those expected, most likely due to the fact that these compounds were the PCDD/F congeners that were present in the raw materials at a significant concentration. In Table 1, total PCDD/F and DL-PCB levels for the fish oils and the different feeds, in pg WHO-TEQ/g, are reported. It is important to point out that, despite Fish Oil B had PCDD/F and PCDD/F+DL-PCB levels clearly above the maximum established, the feeds containing this ingredient in their composition did not exceed the maximum levels for this kind of matrix, with the exception of the total level of PCDD/Fs+DL-PCBs in chicken feed for Treatment 3.

Table 1. Levels of PCDD/Fs and DL-PCBs, expressed in pg WHO-TEQ/g (upperbound values), in fish oils and feeds from the different treatments.

pg WHO-TEQ/g	Fish Oils		Chicken feeds			Rabbit feeds		
	A	B (spiked)	Treatment 1	Treatment 2	Treatment 3	Treatment 1	Treatment 2	Treatment 3
PCDD/Fs	1.95	9.78	0.11	0.39	0.54	0.10	0.15	0.27
DL-PCBs	7.69	19.02	0.48	0.73	1.21	0.23	0.38	0.59
Sum (PCDD/Fs + DL-PCBs)	9.64	28.80	0.59	1.11	1.75	0.33	0.53	0.86

Six different replicates were considered for each of the three separate treatments carried out on the two types of animals selected in this study. Therefore, the results shown in here for each of the cases are mean results from n=6 groups of animals or replicates that were fed with the same feed, during the same period of time and in the same conditions.

In general, congener distribution of PCDD/Fs in chicken samples from the three different treatments resembled in some ways the profile previously observed in the corresponding feeds, this fact indicates that these animals were able to bioaccumulate the 17 toxic PCDD/F congeners. However, it has to be remarked that the percentage of the lowest chlorinated compounds, particularly tetra- and pentachlorinated congeners, was higher in the meat samples than in the feed; whereas for the highest chlorinated congeners (i.e. hepta- and octachlorinated dibenzofurans) the opposite situation was observed. Bioconcentration factors (BCFs), calculated as the ratio between concentration of individual congeners in the meat samples and the feed, were also lower for these highest chlorinated compounds (i.e. BCFs of 1 to 5, in the case of hepta- and octachlorinated dibenzofurans, compared with 8 to 10 for the tetra-substituted PCDD/Fs). The concentrations of the different toxic PCDD/F congeners in the chicken samples, expressed in pg/g fat, are shown in Figure 1. In the majority of cases, the levels increased when increasing the amount of each of the compounds in the feed. Table 2 presents the toxicity related to PCDD/Fs and DL-PCBs, in terms of pg WHO-TEQ/g fat, for chicken and rabbit samples from the three separate treatments. In the case of chicken, it can be observed that, PCDD/F levels in pg WHO-TEQ/g fat were significantly higher in meat from those animals fed a higher contaminated feed, consistently with the concentration increase for the individual congeners.

On the contrary, in rabbit samples a completely different bioaccumulation behaviour was observed compared to the chicken samples. The profile of PCDD/Fs in the rabbit meat did not coincide with that obtained in the feeds. Hepta- and octaclorinated dibenzo-*p*-dioxins contributed largely to the total concentration of PCDDF/s, with relative percentages of 21-23% and 65-68%, respectively. In addition, high BCFs were obtained for these two compounds compared to other PCDD/Fs, being from 34 to 74 for 1,2,3,4,6,7,8-HpCDD and from 29 to 34 for OCDD, respectively. On the other hand, for most of the remaining congeners the levels, expressed in pg/g fat, were close to or below the detection limit of the method. In consequence, as it can be observed in Table 2, there were not significant differences in PCDD/F toxicity, in pg WHO-TEQ/g fat, among rabbit samples from the three different treatments.

For DL-PCBs, the profile was very similar between feeds and meat samples, both for chicken and rabbit from the three different treatments, with the exception of some PCBs that represented less than a 2% of the total DL-PCB concentration in the feeds. As expected, major contribution to the total concentration of DL-PCBs, in pg/g fat, was from PCB 118, which represented 60-65 %, and PCB 105, with a relative percentage of 16-19%. Levels of DL-PCBs, in pg WHO-TEQ/g fat, are shown in Table 2. In the case of chicken samples it can be observed that DL-PCBs contributed up to a 72-82% to the total toxicity of PCDD/Fs and DL-PCBs considered as a group. For rabbit meat the percentage of DL-PCB contribution to the total WHO-TEQ was slightly lower, from 58% to 71% depending on the treatment considered.

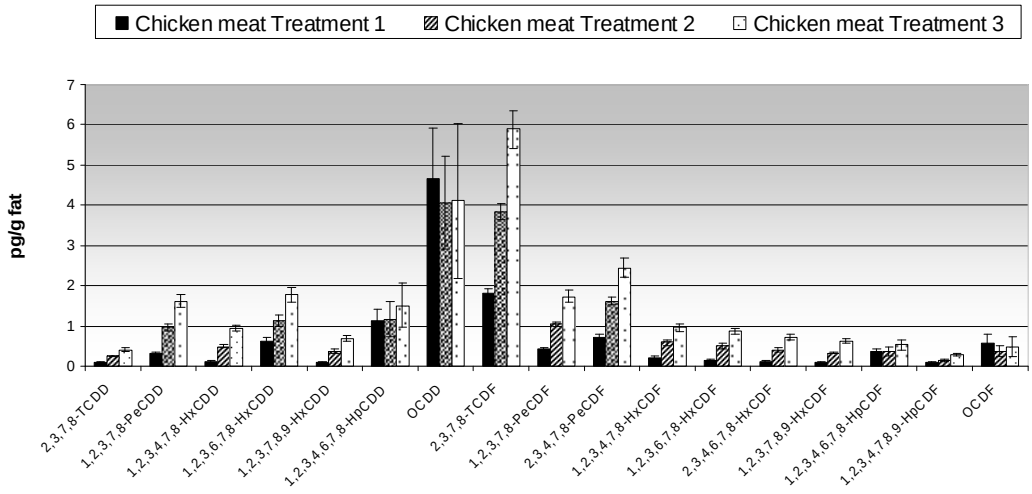

Figure 1. Individual concentrations of PCDD/Fs, expressed in pg/g fat, in chicken meat samples from the three different treatments. Mean values and the corresponding standard deviations (n=6) are represented.

Table 2. Levels of PCDD/Fs and DL-PCBs, expressed in pg WHO-TEQ/g fat (upperbound values), in chicken and rabbit meat samples. Mean values of n=6 replicates are shown. In parenthesis relative standard deviations (RSD%) are included.

pg WHO-TEQ/g fat	Chicken meat			Rabbit meat		
	Treatment 1	Treatment 2	Treatment 3	Treatment 1	Treatment 2	Treatment 3
PCDD/Fs	1.11 (6)	2.85 (5)	4.60 (8)	1.12 (36)	0.86 (15)	0.75 (19)
DL-PCBs	4.92 (7)	8.40 (5)	12.11 (8)	1.56 (18)	1.69 (11)	2.51 (15)
Sum (PCDD/Fs + DL-PCBs)	6.03 (7)	11.24 (4)	16.71 (8)	2.68 (22)	2.55 (7)	3.54 (22)

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