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PCDD/F and DL-PCB levels in meat from broilers and rabbits fed with fish-oil enriched feeds

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ABSTRACT

The aim of this study was to assess the effect on the final levels of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in meat when fats, in particular fish oils, are included as ingredients in feeds. Two types of animals (broilers and rabbits) were fed with three different experimental feeds containing varying amounts of two selected fish oils. PCDD/Fs and DL-PCBs were determined in the fish oils, the feeds and in the animals' meat. For broilers, PCDD/F and DL-PCB profiles in meat samples were similar to those found in the corresponding feeds, even though bioaccumulation of the highest chlorinated PCDD/F congeners seemed to decrease. Depending on the treatment, PCDD/F and the sum of PCDD/F and DL-PCB levels were 1.11–4.60 and 6.03–16.71 pg WHO-TEQ/g fat, respectively. For most of the cases, these values exceeded the maximum established by the Commission Regulation (EC) No. 1881/2006. In contrast, the levels of these contaminants in the corresponding feeds ranged from 0.11 to 0.54 pg WHO-TEQ/g, in the case of PCDD/Fs, and from 0.59 to 1.75 pg WHO-TEQ/g, when DL-PCBs were also included. These levels were, in general, below the maximum allowed by the Commission Directive 2006/13/EC. The results of the experiments with rabbits were not as conclusive as those for broilers although bioaccumulation appeared to be slower.

1. Introduction

Several incidents related to the presence of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in food products have strengthened interest in the study and continuous monitoring of these pollutants in these kinds of matrices. Nowadays, it is well accepted that diet is the main route of human exposure to these pollutants. Depending on habits, more than 90% of PCDD/F and dioxin-like PCB (DL-PCB) background contamination in human beings results from food intake. Moreover, foodstuff of animal origin contributes more to this intake than other products (Liem et al., 2000). In this respect, it has been reported that feed and feed additives for animal growth have been the most frequent entry point of these unwanted substances into the food chain and the cause of several incidents.

In 1997–1998, an increase in PCDD/F levels in milk, butter and meat was detected in Germany in the framework of a surveillance program, and was finally attributed to the use of contaminated citrus pulp as an additive for cow feed (Malisch, 2000). The unexpected presence of PCDD/Fs in minerals currently used as feed ingredients and subsequent incidents have also been reported (Ferrario et al., 2000; Abad et al., 2002; Hoogenboom et al., 2005). In 1999, one of the most important dioxin contamination episodes, the so-called "Belgian crisis", had its origin in the use of a mineral oil as a fat ingredient for the production of feeds for different types of animals (Bernard et al., 1999). More recently, Hoogenboom et al. reported a dioxin incident also related to the use of fat products for feedstuff purposes; in this case a recycled animal fat that was recovered as a slaughter by-product using PCDD/F-contaminated HCl was the origin of dioxin contamination (Hoogenboom et al., 2007).

Some of these incidents were detected as a result of PCDD/F monitoring programs that have been implemented or intensified in several countries in recent years. Moreover, there is increasing concern about the need to monitor different aspects of animal nutrition, in order to preserve animal welfare and to produce safe and good quality products for human consumption at the same time. In this sense, the aims of the "Quality and safety of feeding fats obtained from co-products or by-products of the food chain (feeding fats safety)" project, included in the 6th EC Framework Programme, were related to these topics. One of the main objectives of this project was to identify and quantify undesirable substances, including different families of pollutants (e.g. PCDD/Fs and DL-PCBs), in fats and oils that are co-products or by-products derived from the food chain and that are normally used for feeding purposes.

In the first stage of the project, a wide variety of fats and oils were characterized and PCDD/F and DL-PCB levels were determined (Ábalos et al., 2008). Fish oils contained the highest amounts of both

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PCDD/Fs and the sum of PCDD/Fs and DL-PCBs, with one sample out of nine having levels clearly above the maximum established by the present European Directive related to the presence of these pollutants in animal feed (Commission Directive 2006/13/EC).

The next step in the project dealt with the effects of the use of some of these selected fats as ingredients for the preparation of feeds on animal production. One of the parameters examined was the final levels of PCDD/Fs and DL-PCBs in the meat.

Several studies have been published on the transfer and bioaccumulation of these pollutants in animal tissues after a specified exposure period. Some of these dealt with ruminants and included evaluation of the transfer of contaminants from the feed to the milk (Costera et al., 2006) as well as the levels in different organs (Richter and McLachlan, 2001). Other studies dealt with poultry; for example, Hoogenboom et al. (2004) studied the ratio between Indicator PCB and PCDD/F levels in fat samples from pigs and broilers fed with a 10-fold diluted feed from the "Belgian crisis", followed by a period on clean feed. The authors found lower bioaccumulation of PCDD/Fs and PCBs in fat than theoretically expected, probably due to the lower bioavailability, metabolism, excretion and storage in other tissues (e.g. liver) (Hoogenboom et al., 2004). In 2006, Traag et al. reported a similar study on laying hens, also based on the use of contaminated feed from a diluted sample of chicken feed from the "Belgian crisis" and the effect of an additional period on clean feed following exposure (Traag et al., 2006). Abdominal fat, livers and eggs were collected for analysis. It was concluded that the major part of PCDD/Fs and PCBs were initially stored in the abdominal fat but also that some of the contaminants were transferred to the eggs during exposure. Other studies related to the bioaccumulation of PCDD/Fs and coplanar PCBs in hens fed contaminated feed included data on absorption and excreta and gave information about the concentration distribution in several tissues (Pirard and de Pauw, 2005, 2006). One finding was that the liver seems to be the major storage organ for PCDD/Fs and it tends to accumulate the highest chlorinated congeners in a greater proportion than other tissues.

In the present study the aim was to assess the transfer of PCDD/Fs and DL-PCBs from the fats, used as additives in feed production, to the animal tissues. For this purpose, separate groups of two different types of animals, broilers and rabbits, were fed with three different experimental feeds containing varying amounts 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 used in commercial animal production. Broilers and rabbits were considered for this study since they grow up in a short period of time compared to other animal species, which made possible to carry out the experiments within the length of the project period. In addition, as it has been already mentioned, poultry (e.g. broilers) have been included in several PCDD/F and PCB bioaccumulation studies. On the contrary, scarce information is available on PCDD/F and DL-PCB levels in rabbits (Hamm et al., 2002), despite rabbit meat being regularly consumed in different European countries, for instance it represents approximately 3% of the total consumption of fresh meat in Spain. Finally, fish oils were selected instead of other types of fats since, as stated above, they had previously been found to contain the highest levels of these contaminants among a wide group of different categories of fat by-products or co-products from animal or vegetable origin.

2. Materials and methods

2.1. Animal feeds

Two fish oils were selected and classified as Fish Oil A (low level of contaminants) and Fish Oil B (high level of contaminants), respectively. Fish Oil A was considered since it had levels of PCDD/Fs and DL-PCBs similar to the lowest levels determined for this category of feeding fats in a previous study (Ábalos et al., 2008). In the case of Fish Oil B, in order to raise the levels of these contaminants above the maximum allowed by the European Directive (Commission Directive 2006/13/EC), the oil was spiked with additional known amounts of commercial mixtures of native PCDD/Fs and DL-PCBs (EPA-1613PAR and WP-STK solutions from Wellington Labs., Guelph, Ontario, Canada).

Three feeds for each type of animal (broilers and rabbits) were prepared by adding different percentages of the two selected fish oils to a base mixture composed of raw materials of vegetable origin (e.g. alfalfa, barley, corn, extruded full-fat soybean, soybean meal, sugar beet pulp and sunflower meal). The total percentage of fish oil added to each feed was equivalent to the total fat content usually present in the commercial feeds, that is 6% and 3% of fat in broiler feed and rabbit feed, respectively. The aim was to obtain three different levels of PCDD/F and DL-PCB contamination in the final feeds, giving rise to three different treatments for broilers and three more for rabbits. Thus, for broilers, feed for Treatment 1 (Broiler LC) contained 6% of Fish Oil A, feed for Treatment 2 (Broiler MC) contained a mixture of 3% Fish Oil A and 3% Fish Oil B, and feed for Treatment 3 (Broiler HC) contained 6% Fish Oil B. 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 broiler feeds for each treatment. The three different treatments for the rabbits were coded as Rabbit LC, Rabbit MC and Rabbit HC, respectively.

2.2. Animal studies

Six groups of four broilers (7 d old) and six groups of five rabbits (28 d old) were fed for 40 d and 35 d, respectively, with one of the abovementioned feeds. Each group or pooled sample of four or five animals constitutes a replicate. Thus, 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 to obtain homogeneous samples. In the case of the broilers, the deboned legs including the skin were homogenized. Meat samples were then sent to the laboratory for PCDD/F and DL-PCB determination.

2.3. PCDD/F and DL-PCB analysis

Once received at the laboratory, samples were coded, freeze-dried and re-homogenized again prior to PCDD/F and DL-PCB analysis. Next, samples were spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS, Wellington Laboratories Inc., Guelph, Ontario, Canada) and $^{13}C_{12}$-DL-PCBs (WP-LCS, Wellington Laboratories Inc., Guelph, Ontario, Canada) and then extracted in a Soxhlet for ~24 h with toluene:cyclohexane (1:1) (Merck, Darmstadt, Germany). The extracts were rotary evaporated and kept in the oven overnight (105 °C) in order to eliminate the solvents prior to gravimetrical fat determination. Afterwards, fat residues were redissolved in *n*-hexane (Merck, Darmstadt, Germany). Organic components, fat and other interfering substances were removed by treating the *n*-hexane extracts with sulfuric acid. Then, the extracts were rotary evaporated again down to approximately 1–2 mL approximately and filtered through a PTFE filter prior to the clean-up process.

The purification step was performed by an automated system (Power Prep™, FMS Inc., Waltham, MA, USA). This clean-up procedure is based on solid–liquid adsorption chromatography using a sequential array of three different Teflon prepacked columns of multilayer silica, alumina and carbon adsorbents, respectively