gmp.GALIS: A GLOBAL PROYECT FOR FEED SAFETY. FIRST RESULTS

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Introduction

Galicia is an autonomic region located in the northwest of Spain. It is the second producer of feedingstuffs in Spain, with 2,807,833 ton/year, which represents 13% of total Spanish production[1]. The European laws about food safety: Regulation (EC) nº 178/2002, Regulation (EC) nº 183/2005 and Regulation (EC) nº 882/2004; have enforced higher feed requirements, including production and use when that feed is intended for food-producing animals. These strict new requirements make a difficult task to be achieved by a single producer. Therefore, Galician producers of feedingstuffs, grouped in AGAFAC (compound feedingstuffs producers association), have joined their strengths creating the gmp.GALIS project.
The aim of this contribution is introducing the gmp.GALIS project and it presents the results for PCDDs/Fs and PCBs in feed material during the first four months of work. Final results will be obtained at the end of 2008.

The gmp.GALIS project

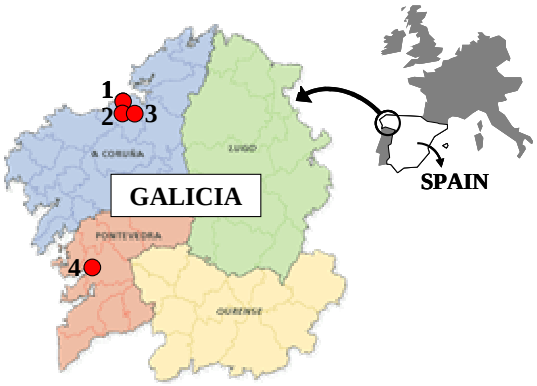
gmp.GALIS project was born with the aim of increasing the safety standards for animal feed in Galicia in agreement with HACCP standards, following already existing models in other countries: GMPT (Nederlands), OVOCOM (Belgium), etc. This project comprises three types of activities:

- Establishment of analytical procedures that guarantee control of contaminants in feed materials.
- Build up a service plan for factories that includes homologation of suppliers.
- To become a leader in animal feeding in Galicia, acting as connection between producers and Administration.

For achieving these objectives two working areas are set:

- gmp.GALIS Lab that coordinates aspects related with analytical laboratories.
- gmp.GALIS Ser that supplies services to producers.

Two kinds of analysis were carried out: Analysis of nutriments: proteins, humidity, ashes, starch and ethereal extract; analysis of pollutants: micotoxins, PCDDs/Fs, PCBs, pesticides and metals. Figure 1 shows the location of the laboratories and analysis made per year. This planning will be kept for four years.



1. University of A Coruña:
- PCDDs/Fs: 40 samples.
- Pesticides: 40 samples.
- Heavy metals: 40 samples.
- PCBs and PAHs.

2. LIGAL:
- Nutriments: 250 samples.

3. CIAM:
- Aflatoxins: 40 samples.
- Micotoxins.

4. Mouriscade Laboratory:
- Nutriments: 650 samples.
- Microbiology: 900 samples.
- Other pathogens.
- Minerals.

Figure 1. Annual planning of analysis and location of laboratories.

Materials and Methods

Analysis of PCDDs/Fs and PCBs

Dry solid samples were ground up to a fine powder and then spiked with labelled PCDDs/F standards (EPA 1613-LCS, Wellington Laboratories, Ontario, Canada) and labelled PCB standards (WP-LCS, Wellington Laboratories, Ontario, Canada) when this analysis is required prior to extraction. Analytes were removed from samples by Soxhlet extraction using toluene for 8 h in a Büchi extraction unit B-811 LSV (Flawil, Switzerland). Extracts were concentrated and the lipid content was determined gravimetrically when it was needed. After that the fat was dissolved in hexane. In the other hand oil and fat samples were dissolved directly in n-hexane after spiking with the same standards. The fats were removed to enable the clean-up by treatment with sulphuric acid[2].

The clean-up was performed on the Power Prep FMS system (Fluid Management Systems, Waltham, USA). The procedure is based on solid/liquid adsorption chromatography using a set of disposable columns: multilayer silica columns, basic alumina columns and PX-21 carbon columns. For PCDDs/Fs analysis a final extract in toluene was recovered. When PCBs are also analysed two extracts were recovered, the first containing the mono-ortho PCB and the second containing the non-ortho PCBs and the PCDDs/Fs. The extracts were concentrated up-to 2 mL using a rotary evaporator (Büchi, Flawil, Switzerland) and the remaining solvent under a gently stream of N_2. Before HRGC/HRMS analysis, samples were rebuilt with the recovery standards: 5 µL of EPA 1613-ISS (Wellington Laboratories, Ontario, Canada) plus 10 µL of nonane for PCDDs/Fs and 2 µL WP-ISS (Wellington Laboratories, Ontario, Canada) plus 25 µL of nonane[3].

PCDDs/Fs analysis was based on US EPA Method 1613 and PCB determinations were based on US EPA Method 1668. A MAT 95 XP coupled to two Trace GC 2000 series gas chromatographs (Thermo Electron, Bremen, Germany) equipped with CTC GC Pal autosamplers (CTC Analytics, Zwingen, Switzerland) was used. Analysis of PCDDs/Fs was performed using a DB-5 capillary column (30 m x 0.25 mm x 0.25 µm; Agilent Technologies, Colorado Springs, Bellefonte, USA) with helium as carrier gas at 1 mL/min in the splitless injection mode (2 µL). The temperature program was from 140 ºC (2 min) to 200 ºC at 11ºC/min, and then to 300 ºC at 3 ºC/min. Analysis of PCBs were performed using a Rtx-2330 (60 m x 0.25 mm x 0.1 µm; Restek, Bellefonte, USA) with helium as carrier gas at 1 mL/min in the splitless injection mode (2 µL). The temperature program was from 80 ºC (2 min) to 225 ºC at 20ºC/min, and then to 300 ºC (5 min) at 2.5 ºC/min. Transfer line temperature was set at 290 ºC in both cases. The mass spectrometer was operated in EI mode (45 eV), using multiple ion detection (MID). Source temperature was set at 260 ºC and the spectrometer was tuned to a minimum resolution of 10,000 (10% valley) using FC-43. The two most abundant isotope peaks (M^+ and $[M+2]^+$ or $[M+4]^+$) of each PCDDs/Fs or PCBs congener were used.

Identification was carried out using chromatographic retention times and isotopic ratios. Quantification was achieved by isotopic dilution method using relative response factors (RRF) obtained by analysis of standard solution mixtures (EPA 1613 CVS solutions; Wellington Laboratories, Ontario, Canada).

Results and Discussion

gmp-GALIS project comprises the analysis of 130 samples for determining PCDDs/Fs along 4 years (2005-2008). From October 2005 to March 2006 eight vegetable (2 gluten, 2 soya, 2 DDG and 1 alfalfa samples), one soyabean oil and four animal fat samples were analysed. These samples are from transport media that supply the factories of Galicia.

Figure 2 shows the results (pg/g) obtained for vegetable and vegetable oil samples. In general low values were obtained and most congeners were at level under 0.1 pg/g. Only congeners that are characteristically higher in this kind of matrix: 1,2,3,4,6,7,8-HpCDD, OCDD and OCDF, presented values above 2 pg/g, with a maximum of 22.2 pg/g for OCDD in one DDG sample. In the case of soyabean oil the HxCDF isomers were important too.

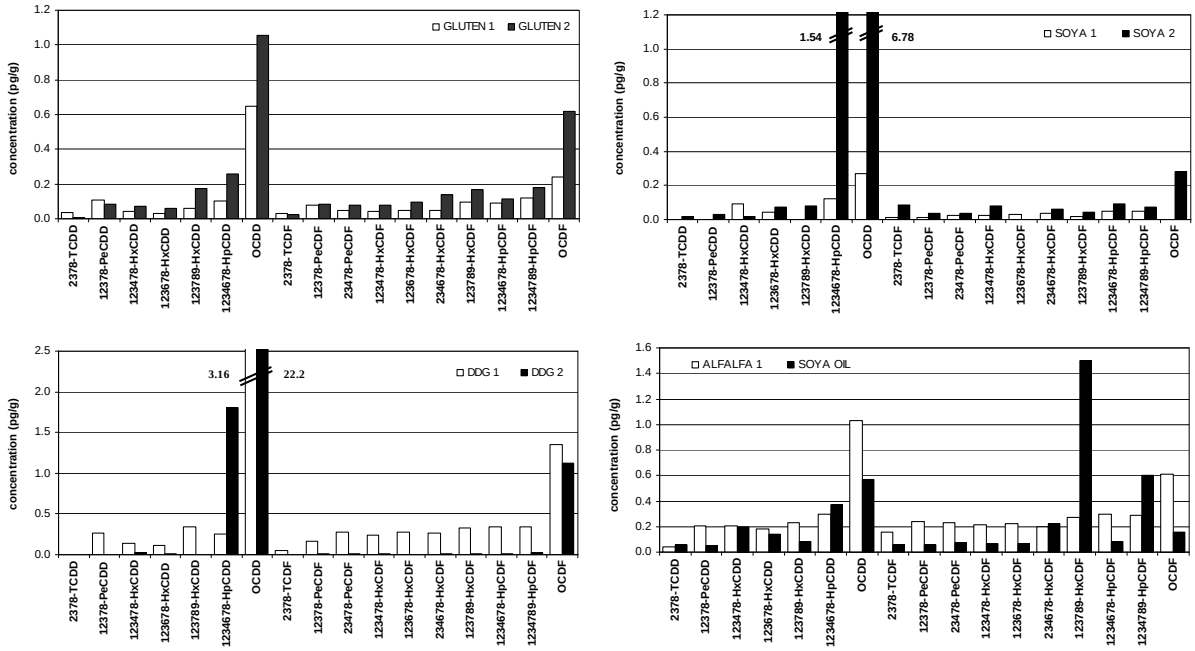


Figure 2. Concentration of PCDDs/Fs congeners (pg/g) in vegetable samples.

Results obtained for animal fat are presented in Figure 3. In general, levels are higher than those obtained for vegetal samples, and almost all congeners are over 0.1 pg/g. This can be due to the lipophilic characteristics of these compounds. HpCDD, OCDD and OCDF were the main compounds again, although *tetra* and *penta* isomers yielded important levels too.

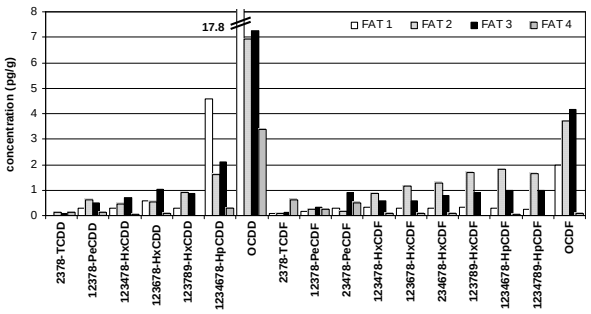


Figure 3. Concentration of PCDDs/Fs congeners (pg/g) in animal fat samples.

The results in terms of WHO-TEQ are presented in Figure 4. All vegetable samples were under the limit (0.75 pg/g) established by Regulation (EC) 2375/2001, and levels were quite low even for soyabean oil. Only the alfalfa sample and one of DDGs samples were over 0.4 pg/g WHO-TEQ. For animal fat all samples were under the 2 pg/g limit.

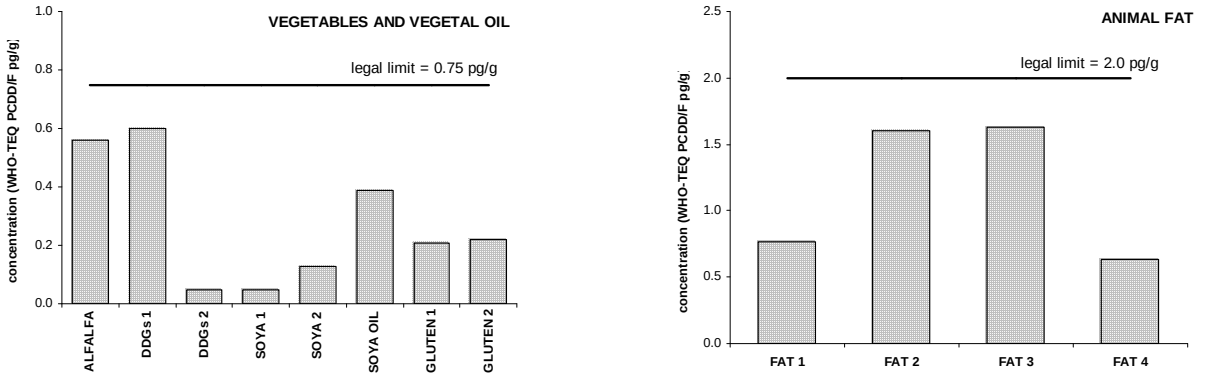


Figure 4. WHO-TEQ values of vegetal and animal fat samples.

PCBs were determined in two samples only. Sample 'DDGs 2' presented a value of 0.005 WHO-TEQ PCBs pg /g what represents a 9% of total WHO-TEQ PCBs+PCDDs/Fs. Sample 'Fat 4' yielded a value of 0.11 WHO-TEQ PCBs pg /g. In this case their contribution to total WHO-TEQ PCBs+PCDDs/Fs represents a 14.86%.
These results are presented as an example because it is too soon to draw conclusions due to the low number of samples analysed.

References

1. Producción de piensos por Comunidades Autónomas 2003. *Instituto Nacional de Estadística*, 2005.
2. Pirard C, Focant JF, De Pauw E. *Anal Bioanal Chem* 2001;372:373.
3. Abad E, Sauló J, Caixach J, Rivera J. *J Chromatog A* 2000;893:383.