Undernutrition combined with dietary mineral oil enhance depuration through fecal excretion of stored dioxin (TCDD) and polychlorinated biphenyls (PCBs) in ewe

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Introduction

Persistent Organic Pollutants (POPs) have deleterious effects on human health (carcinogenic, neurotoxic, reprotoxic, endocrine disrupting effects…). Their high lipophilicity and low degradability properties result in their high level of bioaccumulation, which explain that consumption of food of animal origin is one of the main routes of exposure to POPs for humans [1]. To face to this human health hazard, European sanitary authorities have set maximal regulatory levels for POPs in food of animal origin (Regulation n°1881/2006). Nevertheless, sanitary crises occurred these last decades due to livestock POPs contamination from environmental or accidental exposure [2]. In case of sanitary crisis, POPs depuration process is extremely slow leading to a systematic disposal of the contaminated livestock rather than salvation [3]. There is therefore a real need to find feeding strategies enhancing the depuration of animals in order to reduce the deleterious economic and social damages of sanitary crisis involving POPs. Due to their lipophilic nature, when one strategy aims to depurate livestock from POPs, two combined steps should be considered: i) the POPs release from the adipose tissue (AT) storage compartment to the blood, and ii) the POPs transfer from blood to the fecal excretion compartment. In order to induce the release of polychlorinated biphenyls (PCBs) from AT to blood in ewe, body-fat mobilization induced by undernutrition was recently proven to be an efficient strategy [4]. Moreover, dietary non-absorbable lipid [e.g. mineral oil (MO)] supplementation enhanced POPs fecal output through stimulation of non-biliary excretion from blood to digestive contents, as shown for organochlorine pesticides as hexachlorobenzene in growing lambs [5] and mirex in dairy goats [6]. This study aimed at testing the efficiency of undernutrition combined with MO supplementation as a strategy to decontaminate ewes of stored 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), PCB 126 and PCB 153. The initial hypothesis was that undernutrition combined with dietary MO supplementation would provide synergetic effects on POPs depuration through fecal excretion. Novel aspects of the current study include the fine description at once of POPs toxicokinetic and body lipid dynamic, which is a key step in order to better understand the influence of this latter on lipophilic pollutants fluxes.

Materials and methods

Experimental Design. Nine non-lactating Romane ewes were boxed individually. The 93-day experiment was divided into three successive periods: i) a 27-day exposure period during which ewes were orally exposed to POPs through a spiked concentrate incorporated to a diet covering 82% of ewe maintenance energy requirements (MER: 0.23 MJ net energy/kg body weight [BW]$^{0.75}$/day [7]); ii) a 8-day buffering period during which ewes were fed with a "clean" diet (with non-spiked concentrate) covering 96% of MER; and iii) a 58-day (days 0 to +57) depuration period during which ewes received one of two depuration treatments. Four ewes received a control well-fed (96% of MER) treatment (CTL), while five ewes received an underfed (37% of MER) and MO (liquid paraffin, Codex 68 grade, kinematic viscosity at 40 °C: 60-79 mm²/s, IGOL, Amiens, France) supplemented treatment (UFMO). Diets were composed of 30% barley straw, 30% permanent grassland hay and 40% concentrate for exposure, buffering and CTL treatments, and of 45% straw, 45% hay and 10% MO (dry matter basis) for UFMO treatment. Only non-contaminated concentrate composed the buffering and CTL diets, whereas 25% of total concentrate were non-contaminated and the other 75% were contaminated in the exposure diet. The non-contaminated concentrate was made of 50% dehydrated beet pulp and 50% corn grain, and the contaminated one was made of 48.8% of dehydrated beet pulp, 48.8% corn grain and 2.4% of contaminated rapeseed oil (fresh matter basis) prepared by

mixing 7.0 µg of TCCD (Sigma-Aldrich, Bellefonte, USA), 7.0 µg of PCB 126 (LGC Standards, Molsheim, France) and 7.0 mg of PCB 153 (LGC Standards) in 2.5 kg of commercial food-grade rapeseed oil. This led to oral exposure of 280±35 pg TCDD, 285±35 pg PCB 126 and 281±35 ng PCB 153/kg BW/day over the 27-day exposure period.

Sampling and Chemical Analyses. Ewes were weighed weekly, whereas feces, blood serum and pericaudal subcutaneous adipose tissue (PSAT) were sampled at days 0, +7, +21, +35 and +57 of depuration. Feces (50-150 g) were individually collected straight from rectum, lyophilized, ground through a 1-mm screen and then pooled by treatment based on equal amounts of dry feces from each ewe at each date (n=10; i.e. 2 treatments × 5 dates). Additionally, pools for each ewe (n=9) representative of individual fecal excretion over the depuration period were composited. Blood (9 mL) was collected in tubes with clot activator, maintained at 4 °C during 20 h, before serum was separated by centrifugation and kept at -20 °C. PSAT (3-5 g) was harvested by biopsy, frozen at -20 °C, lyophilized and ground finely. At the end of the depuration period (day+57), ewes were weighed and shorn before they were slaughtered by stunning followed by exsanguination. Gut contents were exhaustively removed from digestive tract, before empty body (whole body including blood minus gut contents and wool) was weighed, frozen at -20 °C, and finally minced, mixed and homogenized using an industrial mixer-grinder. A homogenized 1-kg aliquot was obtained and stored at -20 °C. Total lipids concentration in feces, PSAT and empty body mix was determined according to Folch *et al.* [8], and by means of a commercial kit (HB018, Cypress Diagnostics, Langdorp, Belgium) in serum. Acid-insoluble ash concentrations measured in feedstuffs and feces were used to estimate fecal flows of dry matter [9]. TCDD and PCBs concentrations were determined using gas chromatography coupled with high resolution mass spectrometry (GC-HRMS) according to ISO/IEC 17025:2005 fully accredited methods (except for feces), which have been slightly adapted from previously described methods [10-12].

Statistical Analyses. Data were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS (2003, Cary, USA). For BW, empty body lipids, and TCDD and PCBs burdens, individual data recorded on days 0 and +57 of depuration, as well as changes over this period were analyzed separately with a model including depuration treatment (CTL or UFMO) as fixed effect and ewe as random effect. For serum and PSAT POPs concentrations collected along the depuration period, the model included a covariate term (i.e. individual POPs concentrations on day 0 of depuration), depuration treatment (CTL or UFMO), day and treatment × day interaction as fixed effects, and ewe as random effect. Significance was declared at $P \leq 0.05$, and trends were considered at $0.05 < P \leq 0.10$. Values reported are least square means and standard error of the means (SEM).

Results and discussion

At the beginning of the depuration period (i.e. day 0), estimated empty body burdens accounted for 50±9%, 48±17% and 54±6% of doses orally administrated over the 27-day exposure period, for TCDD, PCB 126 and PCB 153, respectively. Such levels were close to 40-50% absorption rate reported for TCDD in non-lactating cows [13]. Over the 58-day depuration period, UFMO ewes adapted their metabolism in response to undernutrition by mobilizing their body lipid reserves, as characterized by the 21% and 35% decreases in BW and empty body lipids weight, respectively (Table 1). Magnitude of body fatness decrease in response to undernutrition was in broad accordance with previous studies [4, 14].

Compared to day 0 or CTL treatment, fecal POPs concentrations in UFMO ewes increased by 2.1 to 2.5-fold when expressed on dry matter basis. In contrast, they dropped when expressed on lipid basis (Figure 1). This discrepancy is explained by the 7-fold increase in fecal lipids concentration due to UFMO. In accordance, supplementing diets covering MER with MO induced a 2-fold higher mirex fecal concentration in dairy goats [6], and a 2.9-fold higher hexachlorobenzene fecal concentration (fresh basis) in growing lambs [5]. In the present study, we suspect that the POPs transfer rate from the blood to the intestine lumen was the main limiting step of fecal excretion enhancement due to UFMO. This fact may putatively originated from the inability of highly lipophilic molecules such as TCDD, PCB 126 and PCB 153 ($\log K_{ow} > 6.8$) to cross easily through the wall of the intestinal tract by passive diffusion, as previously reported in humans [15].

Decreases in empty body POPs burdens after 58 days of depuration were 2 to 3-fold higher in UFMO ewes than in CTL ewes. Nonetheless, these decreases in UFMO ewes accounted for only 7%, 2% and 6% of the initial burdens of TCDD, PCB 126 and PCB 153, respectively (Table 1). Conversely, the PCBs body burden of growing chickens decreased by 68% after 21 days of undernutrition (50% *ad libitum* level) combined with 10% MO in total diet [16].

Table 1. Body weight, empty body fatness and burdens in TCDD and PCBs of ewes

	Day of the depuration period						Changes over the depuration period			
	Day 0			Day + 57			Day + 57 - Day 0			
	Treatment			Treatment			Treatment			
Item	CTL	UFMO	SEM	CTL	UFMO	SEM	CTL		UFMO	SEM
Body weight (kg)	62	64	3	59	51	6	-3	**	-14	1
Empty body lipids (kg)[a]	12.6	14.7	1.2	11.8	9.8	1.6	-1.0	**	-5.2	0.5
Empty body burden[a]										
TCDD (ng)	247	233	19	239	216	19	-8	*	-17	2
PCB 126 (ng)	220	241	42	227	236	41	+7	**	-6	2
PCB 153 (µg)	258	256	15	253	241	15	-5	**	-15	2

* and ** indicate significant differences between treatment least square means at $P < 0.05$ and 0.01, respectively. [a] Post-mortem measurements for day + 57, or estimated at day 0: for empty body lipids based on decreases in body weight between day 0 and day + 57 and considering -0.38 kg body lipids for -1 kg body weight decrease according to [14]; for empty body burdens based on day + 57 burden + amount of pollutant excreted over the depuration period through feces and wool – amount of pollutant ingested over the depuration period.

Such discrepancy may originate from i) the lower amount of lipid excreted through feces relative to the amount of lipid stored in empty body in ewe than in chicken and ii) the differences in physiological responses to undernutrition between animal models. The slight decrease in POPs body burden (divided by 1.1 on average) combined with the sharp decrease in their dilution pool (i.e. empty body lipids weight divided by 1.5) in response to UFMO treatment, explained that empty body and serum POPs concentrations were in average 1.2-fold higher than for CTL at day +57 (Table 1 and Figure 2). Nonetheless, increases in PSAT POPs concentrations due to UFMO were of higher magnitude (i.e. in average 1.4-fold higher compared to CTL at day +57) than in empty body and serum. Additionally, increases in PSAT POPs concentrations were also observed for CTL along the depuration period, conversely to what was observed in empty body and serum (Figure 2). We suspect that such differential patterns across tissues arise from a delayed redistribution of POPs toward the subcutaneous AT. Indeed, following absorption, POPs first distribute from lymph and blood to the highly-perfused tissues within a few days. Thereafter, a second-step redistribution toward slowly-perfused tissues (like subcutaneous AT) sometimes occurs, as previously observed for highly-chlorinated dioxins in non-lactating cows [13].

Conclusions

In our experimental conditions, undernutrition combined with dietary MO supplementation increased the concentrations of TCDD, PCB 126 and PCB 153 in feces dry matter, compared to CTL. However, the 2-fold magnitude increase was only equivalent to that observed in MO supplemented well-fed small-ruminants [5, 6]. Thus, the present results do not support our initial hypothesis of a putative synergetic effect of undernutrition combined with dietary MO supplementation in ruminants, conversely to previous observations in poultry [16].

When considering fecal and wool excretion as the sole routes of elimination (i.e. metabolism and urinary excretion considered as negligible), a first-order kinetic of depuration applied to empty body burdens at day 0 and +57, allows to derivate TCDD depuration half-lives of 1,200 days for CTL and of 523 days for UFMO. Thus, UFMO strategy may not be benefic unless livestock is contaminated at a level only slightly higher than maximal regulatory limits. In this instance, the reduction of the POPs half-life by a factor of 2 to 3 could make the difference between disposal or salvage of expensive livestock. Such kind of results, combining a fine description at once of POPs toxicokinetics and body lipids dynamics will also be useful in order to calibrate mechanistic models aiming to predict POPs transfer in ruminants [e.g. 17, 18].

Figure 1: Time pattern kinetics of TCDD fecal concentration (pool by treatment) expressed on dry matter (- ◊ -, —♦—) or lipid basis (- □ -, —■—) of CTL (- ◊ -, - □ -) and UFMO (—♦—,—■—) ewes.

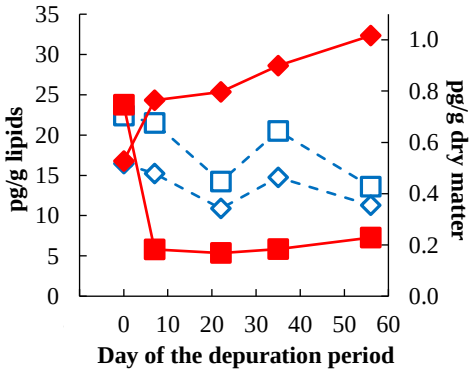
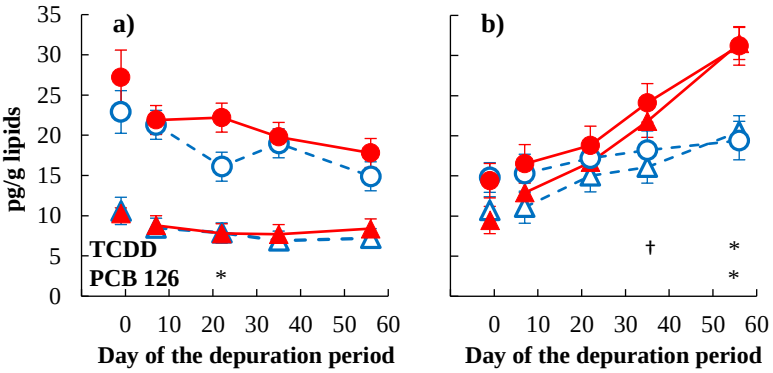


Figure 2: Time pattern kinetics of TCDD (- Δ -, —▲—) and PCB 126 (- ○ -, —●—) concentrations in a) serum and b) pericaudal subcutaneous adipose tissue of CTL (- Δ -, - ○ -) and UFMO (—▲—, —●—) ewes. * indicates a significant ($P \leq 0.05$), and † a trend toward significance ($P \leq 0.10$) for treatment effect in the considering day.



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