EFFECT OF CLENBUTEROL ON LIVER STORES OF DIOXINS AND FURANS IN RATS

Nancy W. Shappell , Lloyd O. Billey, and Vernon Feil[1]

USDA, ARS, Biosciences Research Laboratory, 1605 Albrecht Blvd., Fargo, ND, USA 58105
[1]Retired from same.

Introduction

This study reports the effect of clenbuterol, a proven leanness-enhancing agent for meat producing animals, on liver stores of dioxins and furans. While we previously reported that clenbuterol was successful in lowering the body burden of these compounds in the fat of rats,[1] the effect on liver stores was unknown. Possible effects on the liver included lowering congener stores via bodily elimination or increasing congener stores as a result of accumulation from mobilized fat stores. Either effect would be beneficial in a remediation sense. If the decrease in liver congener concentrations was great enough, then livers could be used in the food supply. If instead, body stores of congeners are mobilized and then accumulated in the liver, this would reduce total contaminated tissue, which would allow for more economical disposal (liver versus whole carcass).

Materials & Methods

The samples analyzed in this experiment were from animals used in previously published work.[1] Male Sprague Dawley rats (32) were grouped in replicates (4) based on weight gain and feed intake over a one week period and randomly assigned to treatment groups within replicates. For ten days 16 rats received ground feed laced with corn oil (100ul on 3g ground rat chow) containing dioxin and furan congeners [doses and toxic equivalency factors (TEFs) listed in Table 1] while 16 rats were given feed with corn oil only. On day 11 rats were subgrouped (eight animals) and fed rat chow with or without 2mg clenbuterol / kg feed for 16 days. At necropsy, tissue and organ weights were recorded (4 experimental groups, n=8). EPA Method 1613 was used for livers from dioxin-dosed rats,[2] while undosed livers were ground with celite (2:1 celite to tissue) and then extracted using methylene chloride:hexane (1:1) at 100°C on an Accelerated Solvent Extractor (ASE Dionex, Sunnyvale, CA, results were confirmed as equivalent to the EPA Method). Due to expense, congener analysis was done on only one liver per treatment replicate (n=4). ASE extracts were processed on a Dioxin-Prep™ system (Fluid Management Systems, Waltham, MA), followed by high resolution GC-MS analyses. Clenbuterol effect on congener data was analyzed using a mixed model analysis of variance (SAS/STAT, SAS Institute, Cary, NC) with dixoin-dosed and undosed groups analyzed separately. Clenbuterol treatment was the independent variable and replicate was used as a blocking factor.

Results & Discussion

We previously reported that clenbuterol reduced body fat by 28% and increased muscle mass by 25%, liver weights were reduced by 15% when expressed as a % of body weight minus gastrointestinal tract [BWT-GI] or 7% on absolute liver weight, Fig. 1.[1]

Table 1. Dose, TEF Values, and % Dose Retained in Liver

Congener	Total Dose (in 10 days)	TEF	% Dose Retained in Liver (+/- Clenbuterol)	
2,3,7,8 TCDF	6 ng	0.1	1.0 / 0.6	(p<.02)
2,3,4,7,8 PeCDF	10 ng	0.5	50.0 / 47.9	
1,2,3,4,6,7,8 HpCDF	6 ng	0.01	20.4 / 20.2	
OCDF	27 ng	0.001	2.7 / 2.7	
1,2,3,7,8 PeCDD	3 ng	0.5	11.3 / 13.4	
2,3,7,8 TCDD	3 ng	1.0	22.5 / 25.8	
1,2,3,6,7,8 HxCDD	13 ng	0.1	38.2 / 42.5	
1,2,3,4,6,7,8 HpCDD	27 ng	0.01	12.8 / 12.4	
OCDD	27 ng	0.001	3.6 / 3.7	

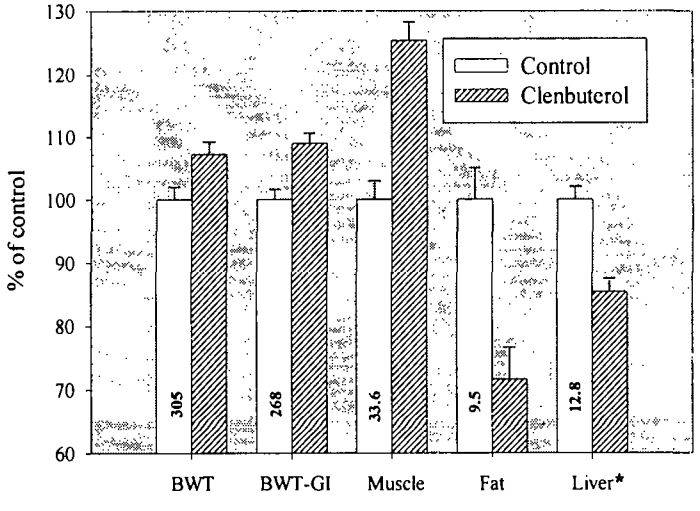

Figure 1. Effect of clenbuterol on tissue/organ weights.[1] PCDD/Fs were without effect on these tissues and organs; therefore, control and PCDD/Fs treated rats vs clenbuterol and PCDD/Fs /clenbuterol treated rats are presented (LS Means ± S.E., n=8). All tissue weights from clenbuterol treatment were different (p≤.002, *indicates analysis as % BWT-GI). Values on bars represent absolute grams of tissue, mean clenbuterol liver weight = 11.9.

Clenbuterol treatment tended to increase the concentration of PCDD/Fs in liver of rats whether dosed with exogenous PCDD/Fs or not (Fig. 2). The increases reached significance in five out of nine congeners (p≤.10) in rats that received clenbuterol only, no PCDD/Fs (Panel A). Increases for these congener concentrations ranged from 14 – 45%, some greater than would be expected by the 7% decrease seen for liver weight with clenbuterol treatment. One conclusion that could e drawn is that the PCDD/Fs released from mobilized fats stores were not excreted, but in fact redistributed to the liver. Significant increases in liver congener concentrations were not observed with exposure to PCDD/Fs (Panel B). The one exception to the trend for increasing congener concentrations was

TCDF in PCDD/Fs-treated rats, where a 39% decrease was found in clenbuterol-treated rats (Panel B). This is consistent with results seen in fat,[1] indicating a rapid metabolism and excretion.

Clenbuterol significantly increased the total endogenous liver burden of PCDD/Fs of one congener: 1,2,3,6,7,8 HxCDD (~ 23%, Fig.3, Panel A). In contrast, clenbuterol reduced liver burdens in rats dosed with PCDD/Fs of one congener: 2,3,7,8 TCDF (39%, Panel B, also as % dose retained Table 1). The fat burden of this same congener was also reduced (66%).[1] These results are consistent with literature reports of extensive metabolism[3] and short half-life[4] for 2,3,7,8 TCDF.

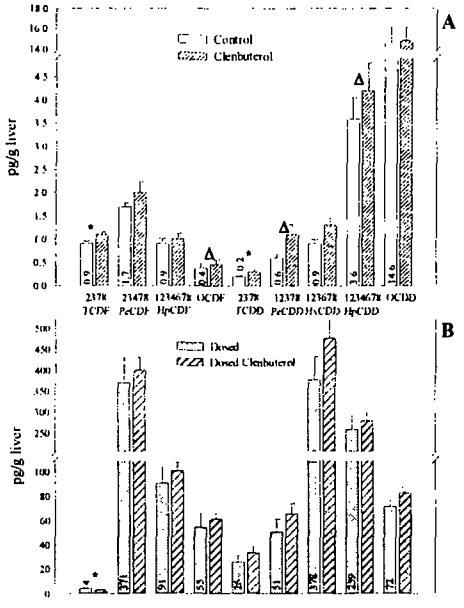

Figure 2. Effect of clenbuterol on PCDD/Fs concentration in liver. Means ± S.E. (n=4).

A) Control vs clenbuterol-treated rats, means) differ at p≤.05 for * and p≤.10 for Δ.

B) Dosed vs dosed/clenbuterol-treated rats, means differ at p≤.05 for *.

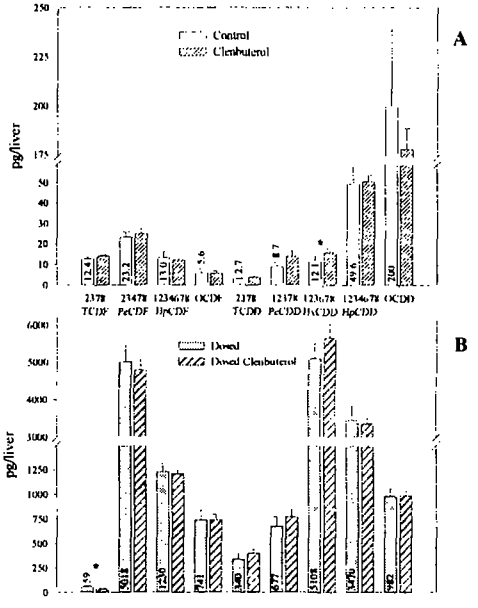

Figure 3. Effect of clenbuterol on PCDD/Fs burden in liver. Means ± S.E. (n=4).

A) Control vs clenbuterol-treated rats, means differ at p≤.05 for * and p≤.10for Δ.

B) Dosed vs dosed/clenbuterol-treated rats; means differ at p≤.05 for *.

One way of assessing shifts in PCDD/Fs body burdens is by looking at the ratio of PCDD/Fs in liver and fat (Figure 4.) Our results are consistent with those found in rats, marmosets, and humans as summarized by Van den Berg et al.,[5] indicating liver versus fat deposition increases as

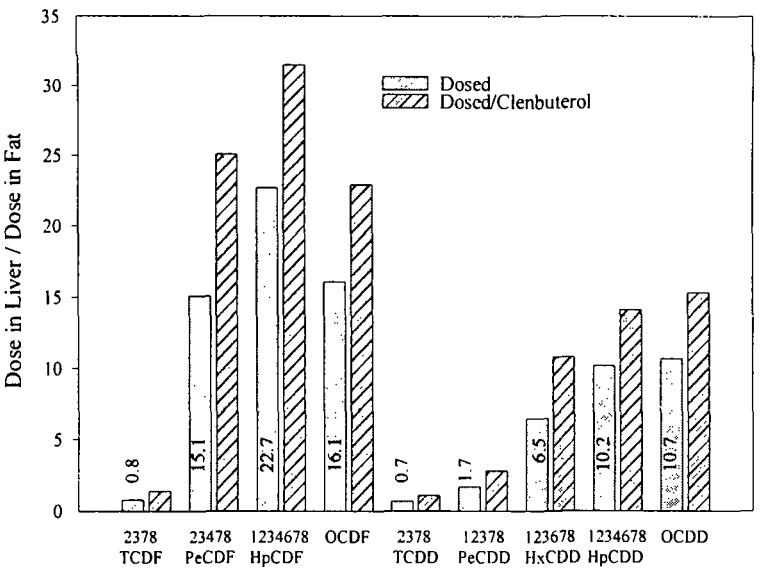

Figure 4. Effect of clenbuterol on liver to fat ratio of PCDD/Fs. (These ratios reflect an inherent overestimation, as total fat is based on grams dissected, not total body fat.)

congener chlorination number increases. These ratios indicate that clenbuterol has shifted PCDD/Fs burden in the direction of the liver (increasing from 38-66%), an advantage for remediation purposes. The effect of clenbuterol on muscle stores of PCDD/Fs is currently being evaluated. Thus far, because clenbuterol decreased fat and PCDD/Fs in fat, it would appear it may have promise for use as a tool in remediation of dioxin/furan contaminated livestock.

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