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

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Introduction

Dioxins have been found as contaminants in beef and chicken in the United States. Contact (rubbing or chewing) with pentachlorophenol treated lumber has been implicated as the source of dioxin contamination in beef [1], while contaminated Ball clay used as an anti-caking agent in feeds was confirmed as the source of poultry contamination[2]. In Belgium, millions of chickens were destroyed after having eaten feed contaminated with dioxins, resulting in an estimated cost of $900 million (chemical analyses, lost revenues, and removal of contaminated products). Few methods have been explored for remediation of animals exposed to dioxins. This study reports the efficacy of using the leanness enhancing agent clenbuterol in remediation of dioxin-contaminated animals was investigated. If a feeding regimen using clenbuterol was successful in lowering dioxins, millions of dollars could be saved and a tainted food supply salvaged.

Materials & Methods

Male Sprague Dawley rats (32) were grouped in replicates (4) based on weight gain and feed consumption 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 (100µl 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. After laced chow was consumed, *ad libitum* access to untreated ground feed was provided for a seven hour period. On day 11 rats were subgrouped (eight animals) and fed rat chow +/- 2mg clenbuterol / kg feed. The corn oil-supplemented feed was provided during this period because a preliminary experiment found feed intake changed when oil was removed from diet. Daily feed intake and body weights were recorded. At necropsy, tissue and organ weights were recorded and fat (epididymal, renal, and abdominal) dissected for dioxin analysis[3]. Polychlorinated dibenzo-*p*-dioxins/furans (PCDD/Fs) values were tested for statistical significance separately for each level of PCDD/Fs by t-test. Other parameters were tested using a two-way mixed model analysis of variance (ANOVA).

Results & Discussion

Clenbuterol reduced body fat by 28% and increased muscle mass by 25% (Fig. 1, $p \leq .002$). Liver, testes, kidneys, and lungs weights were ~8% lower in clenbuterol-treated rats compared to controls, when expressed as a % of body weight minus gastrointestinal tract ([BWT-GI],

Table 1. Dose & TEQ Values

Congener	Total Dose (in 10 days)	TEF
2,3,7,8 TCDF	6 ng	0.1
2,3,4,7,8 PeCDF	10 ng	0.5
1,2,3,4,6,7,8 HpCDF	6 ng	0.01
OCDF	27 ng	0.001
1,2,3,7,8 PeCDD	3 ng	0.5
2,3,7,8 TCDD	3 ng	1.0
1,2,3,4,6,7,8 HpCDD	27 ng	0.01
OCDD	27 ng	0.001

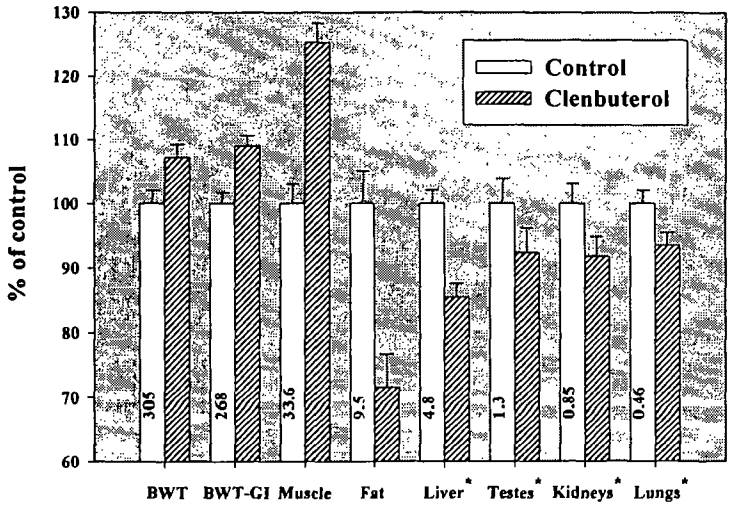

Figure 1. Effect of clenbuterol on tissue/organ weights. PCDD/Fs were without effect on these tissues and organs, therefore control and PCDD/Fs treated rats vs clenbuterol and PCDD/Fs /clenbuterol treated rats are presented (LS Means ± S.E., n=8). All tissue weights from clenbuterol treatment were different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

$p \leq .002$). These findings are consistent with literature[4]. An interaction of treatments was found on heart and spleen weights when analyzed as % BWT – GI. PCDD/Fs increased heart weight 7%, while clenbuterol or PCDD/Fs / clenbuterol treatment increased weights by 11-12% ($P \leq .01$). Spleen weight increased with PCDD/Fs treatment (12% ($p < .05$, data not shown), but the effect was ablated by clenbuterol.

Clenbuterol treatment had little effect on PCDD/Fs concentration in fat (Fig. 2). Concentrations of seven out of nine congeners tended to increase in rats that received clenbuterol only (no exogenous PCDD/Fs dosing, controls, Panel A) but only OCDF and OCDD were significantly

different (increasing 33% p≤.05 and 36% p≤.07, respectively). These same seven congeners tended to increase in rats that received clenbuterol after PCDD/Fs treatment, but 2,3,7,8 TCDD and 1,2,3,7,8 PeCDD were the two congeners that differed significantly (increasing 16% p≤ .06 and 13% p≤.04 , respectively; Panel B).

The endogenous burden of PCDD/Fs in fat was decreased ~ 24% by clenbuterol in the case of two congeners examined: 2,3,7,8 TCDF and 2,3,4,7,8 PeCDF (Fig. 3 A, p≤.02). It is possible that clenbuterol may have reduced the other congeners, but the fact that undosed congener levels were close to the limits of detection may have prevented such assessment. The sum of the resultant toxic equivalency (TEQ) value in fat was reduced 22% by clenbuterol treatment (Table 2).

In rats dosed with PCDD/Fs, clenbuterol was effective in reducing every dosed congener burden in fat by ~ 30% with the exception of 2,3,4,7,8 PeCDF and 2,3,7,8 TCDF, which were reduced by 43% and 66% respectively (Fig.3 B). Clenbuterol's ability to reduce congener burden in fat after congener dosing was correlated to reduction in body fat, as dissected fat decreased 28%.
The resultant TEQ reduction in fat was 43% due to clenbuterol treatment (Table 2). Extension of the clenbuterol treatment period may result in an even greater reduction in congener burden, and requires further study.

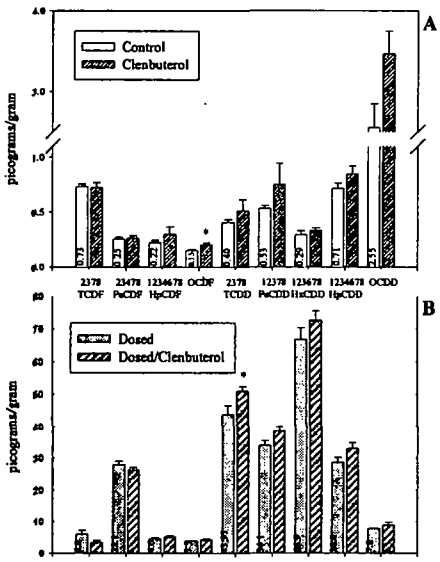

Figure 2. Effect of clenbuterol on PCDD/Fs concentration in fat. Means ± S.E (n=4) **A)** Control vs clenbuterol treated rats, means differ at p≤.05 for *, and p≤.07 for Δ. **B)** Dosed vs dosed/clenbuterol treated rats, means differ at p≤.04 for *, and p≤.06 for Δ.

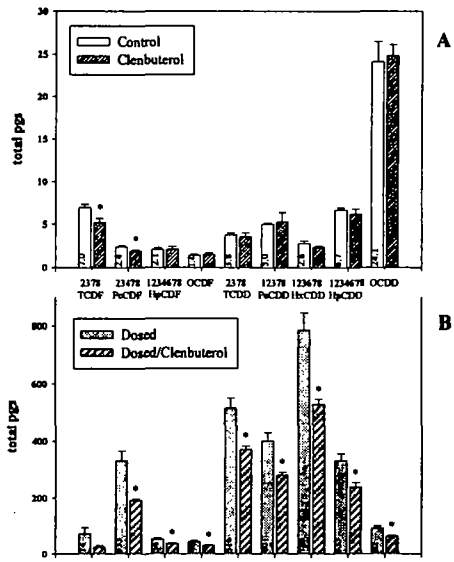

Figure 3. Effect of clenbuterol on PCDD/Fs burden in fat. Means ± S.E (n=4) **A)** Control vs clenbuterol treated rats, means differ at p≤.02 for *. **B)** Dosed vs dosed/clenbuterol treated rats, means differ at p≤.02 for *, and p≤.09 for Δ.

The concentration of congeners in other tissues is currently being evaluated. Although the mechanism for elimination of these contaminants has not been elucidated, if these results can be repeated in food animals, clenbuterol may be effective as a tool in remediation of dioxin/furan contaminated livestock.

Table 2. TEQs per total fat

Treatment	TEQs (mean)	S.E.
Control	1.96[a]	.036
Clenbuterol	1.52[b]	.100
Dosed	177[c]	18.1
Dosed/Clenbuterol	100[d]	3.2

Values with different letters are different ($p \leq .02$).

Acknowledgements

The authors would like to acknowledge the input of the Animal Metabolism Unit's scientists in project discussions: Drs. G. Larsen, H. Hakk, J. Huwe, D. Smith, and W. Shelver; Dr. Huwe's preparation of the furan/dioxin dose, excellent technical assistance of Jean Picard and Kristin McDonald in sample extraction, tireless tenacity of Richard Zaylskie for HR-GC-MS analyses, and Marge Lorentzsen for review of GC-MS data. The use of trade, form or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

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