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Low-dose contamination of feed with ractopamine hydrochloride and resulting tissue residue concentrations
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Introduction
Due to developing meat trade issues associated with use of the beta-agonist ractopamine hydrochloride (RH) in livestock production, this project was designed to better understand the implications of low-dose contamination of feed with RH in beef cattle diets.

Methods
In order to determine the potential RH residues in tissues from beef cattle subjected to small amounts of RH contamination of feed, 35 steers were placed in individual pens and their diets were mixed by hand daily. Tallow, contaminated at very low levels, was added to ensure proper dietary requirements were met. The treatments (fed for 100 days total) were as follows: 100% conventional tallow, 66% conventional tallow, 33% conventional tallow, 100% natural tallow, and 100% natural tallow plus 10 days of conventional tallow at the end of the feeding period to further investigate low-dose contamination when fed almost entirely natural tallow. In other words, both conventional and natural tallow were sourced from a rendering facility and were used to mix treatments (i.e., the 66% conventional tallow treatment was 66% conventional and 34% natural tallow, etc.). Low levels of RH contamination in conventional tallow, using liquid chromatography mass spectrometry (LC-MS) quantitation, ranged from 3 to 32 ppb. Muscle, liver, subcutaneous fat, kidney, and abomasum samples were colleted at a commercial beef harvest facility for evaluation of residues.

Upon collection, samples were placed in sterile whirl-pak bags in direct contact with ice to prevent conversion of ractopamine glucuronides into ractopamine (Ulrey et al., 2013). Samples were then transported to Colorado State University (Fort Collins, CO, USA) and stored in a -20°C freezer until processing. Samples were processed via cryogenic freezing in liquid nitrogen, followed by homogenization, and then were extracted and prepared for LC-MS. Peak picking/integration was performed using Quanlynx software. Quantification of samples and QCs was performed using linear regression against an external standard curve. Separate standard curves were generated for each sample type.

Parent ractopamine and total ractopamine (ractopamine + ractopamine glucuronides) concentration in each tissue type were analyzed separately using a general linear mixed model. The model included fixed effects of tissue and RH inclusion. The effects identified within individuals were reported using individual steer as the experimental unit. Back-transformed least squares means, standard errors, and 95% confidence intervals were computed for each tallow inclusion level and each tissue. Additionally, differences in least squares means between treatment gorups were computed.

Results
Parent and total RH quantified in all tissues were either non-detectable, below the limit of quantitation (LOQ), or barely above the LOQ.

For muscle tissue, regardless of treatment, all samples for both parent and total RH were non-detectable [limit of detection (LOD): 0.06 ppb].

In liver tissue, most samples were non-detectable (LOD: 016 ppb), and only four samples total were above the detection limit. Two samples were below the LOQ, making it hard to determine whether the values were accurate. The highest detectable level of RH in liver tissue was 8 ppb (from the 66% conventional treatment), which is still far below the maximum residue limit (MRL) set by the Codex Alimentarius Commission for liver (40 ppb). In subcutaneous fat, which was collected from kidney, pelvic, and heart fat, and in kidney samples, all treatments resulted in parent and total RH residues below detection (LOD: 0.23 ppb or 0.06 ppb, respectively) or below the LOQ (0.93 ppb or 0.2 ppb, respectively). Abomasum samples followed much the same trend, with 83% and 66% of samples below the LOQ (0.2 ppb) for parent and total RH, respectively. Furthermore, two samples were below the LOD (0.06 ppb), while the remaining samples ranged from 0.21 to 4.22 ppb.

Conclusion
The current study is particularly important because of the global trend toward zero-tolerance policies for ractopamine residues, regardless of tissue type and regardless of current MRLs. It is critical that data are collected to delineate the impact of potential low-dose contamination of feed with RH because detection of measurable residues will continue to be problematic even in cattle not receiving RH in their diet. While current regulatory standards are based on parent rather than total ractopamine, import decisions by some entities are now based upon total measures, which makes meeting zero-tolerance requirements even more challenging. Results from this study suggest that there is a very small likelihood of tissues testing positive for RH as a result of low-dose contamination of the diet in those cattle that do not necessarily receive RH in their daily rations. There is a major lack of scientific evidence supporting trade barriers associated with RH residues, but it is nonetheless crucial to understand how RH behaves in tissues when animals are subjected to minor amounts of the compound.

Notes