RACTOPAMINE WITHDRAWAL IN YEARLING STEERS: SAMPLING AND RESIDUE TESTING OF REGULATORY TARGET AND OFF-TARGET TISSUES

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I. INTRODUCTION

Due to developing meat trade issues associated with use of ractopamine hydrochloride in livestock production, this study was conducted using steers enrolled in a feedlot study to assess the impact of imposing withdrawal procedures from ractopamine hydrochloride (HCl) during cattle finishing. The objective was to determine concentrations of both parent ractopamine (marker residue for regulatory monitoring purposes) and total ractopamine (ractopamine + ractopamine glucuronides) residues in different tissues from steers fed ractopamine hydrochloride at a commonly used dose and duration and at various withdrawal times of 12 hours, 2 days, 4 days, and 7 days. Liver (regulatory on-target tissue) and abomasum (off-target tissue) tissues were collected at harvest.

II. MATERIALS AND METHODS

Six pen blocks (30 pens) of yearling steers, totaling 1,500 head, were fed and harvested in a balanced design according to the withdrawal times (Study FRR1708). Samples (at least 100 g) of the tissues were collected from two animals per pen of treated animals (for a total of 12 animals per treatment group) and one animal per pen for control (untreated) animals (for a total of six animals). Tissues were collected aseptically, within animal, after commercial processing. Upon collection, samples were placed in sterile Whirl-Pak bags and placed in direct contact with ice to prevent conversion of ractopamine glucuronides into ractopamine [1]. Samples were then transported to Colorado State University (Fort Collins, CO) and stored in a -20°C freezer until processing. Ractopamine concentrations in processed tissue samples were then determined by a multiple reaction monitoring assay performed using ultra performance liquid chromatography coupled to tandem quadrupole mass spectrometry (UPLC-MS/MS). The method, developed and validated at Colorado State University for each tissue type, was adapted from the AOAC official method 2011.23. Peak picking and 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.

Ractopamine and total ractopamine concentrations in each tissue type were analyzed separately using a general linear mixed models approach. Analysis of variance included fixed effects of tissue and withdrawal time, with a random effect of block.

III. RESULTS AND DISCUSSION

Mean parent and total ractopamine residues in liver were 3.40 and 3.53 ppb, respectively, in control cattle (those not fed ractopamine HCl; Table 1). Liver samples from treated cattle were only collected after 2, 4, and 7 days of withdrawal from ractopamine HCl because data are already available regarding residues at 12-hour withdrawal. The highest total ractopamine level was detected at the initial collection timepoint (2-day withdrawal; Table 1). After a 2-day withdrawal period, the mean total ractopamine residue in liver was 8.60 ppb; however, following 4 and 7 days of withdrawal, residues were reduced to 3.96 and 3.59 ppb, respectively (Table 1). Parent and total ractopamine were detectable in all abomasum samples, including untreated

controls, with the highest presence at 12-hour withdrawal (Table 2). Because concentrations were above zero for control samples, as well as 7-day withdrawal samples, feed tallow samples were analyzed. In analyzed tallow samples, parent and total ractopamine concentration varied greatly from 0.40 to 50.80 ppb, potentially explaining the lack of negative controls and the fact that withdrawal did not result in non-detectable levels.

Table 1 Mean ractopamine residues in liver tissue collected from cattle, fed ractopamine hydrochloride according to label, at different withdrawal times*

Treatment	Parent Ractopamine (ppb)	Total Ractopamine (ppb)
Control	3.40	3.53
2 days	3.48	8.60
4 days	3.44	3.96
7 days	3.43	3.59

* Cattle in the control treatment group did not receive ractopamine hydrochloride.

Table 2 Mean ractopamine residues in abomasum tissue collected from cattle, fed ractopamine hydrochloride according to label, at different withdrawal times*

Treatment	Parent Ractopamine (ppb)	Total Ractopamine (ppb)
Control	4.27	4.28
12 hours	16.59	20.05
2 days	5.29	5.80
4 days	4.52	4.61
7 days	4.33	4.35

* Cattle in the control treatment group did not receive ractopamine hydrochloride.

IV. CONCLUSION

This study is of particular importance because residue testing of off-target (for regulatory MRL purposes) tissues has increased. The current maximum residue limits (MRL) for liver according to Codex Alimentarius and tolerance for the FDA are 40 and 90 ppb, respectively [2]. For muscle, the MRL/tolerance are 10 and 30 ppb, respectively [2]. Because liver and muscle are regulatory target tissues, meeting these regulatory limits is critical to ensure food safety. Ractopamine concentrations for all of the liver samples were below both Codex and FDA limits. Several of the 12-hour withdrawal abomasum samples (off-target tissue) were above the Codex MRL for muscle (10 ppb) and one was above the MRL for liver (40 ppb), which can be a marketing challenge as some markets apply the muscle MRL to off-target tissues. Although regulatory limits are based on parent rather than total ractopamine, some countries, such as China, are now basing import decisions on measures of total ractopamine concentrations. For this reason, both consumption data and depletion curves are necessary, making this study highly applicable to industry. Additionally, ractopamine was detected in samples from cattle fed in the negative control (untreated) group, which presented a major challenge; cross-contamination likely occurred at some point (e.g., in feed tallow) during production or harvest. Further studies should focus on determining and limiting sources of cross-contamination.

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