Depletion of penicillin G residues in sows after intramuscular injection

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Abstract
A penicillin G procaine residue depletion study was conducted in heavy sows to estimate the pre-slaughter withdrawal periods necessary to clear penicillin from kidney and muscle. Heavy sows (n=126) were treated with penicillin G procaine at a 5x dose (33,000 IU/kg) for 3 consecutive days by intramuscular (IM) injection using 3 separate patterns of drug administration. Treatments differed by pattern and volume of penicillin G procaine administration. Sets of 6 animals per treatment were each slaughtered with 5, 10, 15, 20, 25, 32, and 39 day withdrawal periods; skeletal muscle and kidney were collected for penicillin G analysis by LC-MS/MS. Penicillin residues in skeletal muscle averaged 23.5 ± 10.5 ng/g at withdrawal day 5 for all treatments, but averaged 3,760 ± 1,930 ppb in kidney. By 15 days of withdrawal, skeletal muscle penicillin G residues were quantifiable in only one of 18 (5.5%) treated hogs (3.4 ng/g) but were easily detected in kidneys of 50% of the treated hogs, with kidney residues in all hogs averaging 119 ± 199 ng/g (mean includes 8 non-detects counted at ½ the limit of detection). Using an action limit of 25 ng/g and a ln-linear depletion model, the withdrawal period required for penicillin depletion in muscle was 13 days, whereas a 52-day withdrawal period was required for kidney. The FARAD recommended withdrawal period of 15 days for hogs treated with extra-label doses of penicillin is adequate for skeletal muscle, but is inadequate for kidney. Slaughter of penicillin treated hogs after a 15-day withdrawal period, with kidney discard into inedible offal would ensure the human food safety of skeletal muscle.

Introduction
Penicillin in its various forms has been used by the swine industry for decades; during this period penicillin’s use has not typically been associated with violative drug residues. Since 2011, however, detections of penicillin residues in sow tissues by the Food Safety and Inspection Service (FSIS) have increased substantially resulting in a number of carcass condemnations and the risk that swine producers may be ‘blackballed’ from packing plants. A major change in the regulatory framework for penicillin detection has been FSIS’s employment of the new Charm-KIS test kit in place of the “FAST” swab test for screening animal tissues for penicillin residues (FSIS, 2011a). Currently the US FDA has not established a tolerance set for allowable penicillin residues in edible tissues of pork.

Sows are typically treated with about 5 times the label penicillin G procaine dose that is used for growing swine. Producers, with the advice and consent of a consulting veterinarian, can legally use this dose in an “off-label” manner provided a proper pre-slaughter withdrawal time is selected. Unfortunately, few data exist which describe the depletion of penicillin residues from sow tissues under such use conditions. Apley et al. (2009) conducted a residue depletion trial in which heavy sows were dosed with a single 5x penicillin G procaine dose, but pre-slaughter withdrawal periods selected (2, 4, 6, and 8 days) were too short to properly model penicillin G residue depletion. For example rates of penicillin detection after an 8-d withdrawal period were 60, 80, and 100% in kidney, muscle, and injection sites, respectively; in the same tissues, frequencies of quantifiable residues were 40, 40, and 100% respectively. Because penicillin G procaine depletion rates are poorly defined, a residue depletion study was conducted to provide withdrawal period estimates for sows treated with extra-label doses of penicillin G procaine.

Material and Methods
The North Dakota State University (NDSU) Animal Care and Use Committee approved a detailed protocol prior to the initiation of the study. Heavy sows (n = 160) were purchased from the North Dakota Pig Cooperative (Larimore, ND) and were group housed in concrete-floored pens with straw bedding. Animals were group fed a corn-soybean ration daily; pens were cleaned daily and water was available on an ad libitum basis. Animals that were lame, which had visible abscesses or other visible anomalies were not included in the study. Within the pool of healthy animals, sows were randomly allocated to pen and treatment (injection pattern) so that each pen contained 21 sows. Within treatment, sows were randomly allocated to a post treatment withdrawal slaughter day. Treatments, which differed in the injection volume and pattern, are not defined here because there was insufficient evidence to infer that treatment had a discernible effect on residue depletion.

Sows (n = 126) were weighed and penicillin G procaine (Norocillin; Norbrook Laboratories, Lenexa, KS; 33,000 U/kg BW) was administered via intramuscular injection through 3.8 cm, 16-gauge needles for three consecutive days. Sows were killed after 5, 10, 15, 20, 25, 32, or 39 day withdrawal periods relative to the last dosing day. Kidney and skeletal muscle (mid portion
of the longissimus dorsi) were collected. Samples were placed on dry-ice and subsequently stored at -80 °C until analysis.

Tissues were processed by homogenization on dry ice to prevent thaw and degradation of penicillin residues. Kidney and muscle samples were extracted using the FSIS method CLG-BLAC.02 with some modifications (FSIS, 2011b). Blank (negative control) and fortified samples (spiked with 25 ng/g penicillin G procaine) were extracted with each sample set in duplicate. Trial samples were extracted in duplicate by withdrawal day. Before analysis, a deuterated internal standard (Penicillin G-d7; Sigma Aldrich, St Louis, MO) was added to each sample extract at an end concentration of 100 ng/mL. Blank sample extracts were utilized to prepare matrix matched standard curves containing 2 to 500 ng/mL of penicillin G. A fresh standard curve was made for each sample set and samples and curves were analyzed within 24 hours of preparation. Apley et al's. (2009) analytical method was modified slightly for penicillin analysis using a Waters (Milford, MA) Ultra High Performance Liquid Chromatograph coupled to a tandem quadrupole mass spectrometer. The detection method was modified to monitor additional fragment ions for Penicillin G to improve confirmation and quantification of residues. Reported data are not corrected for recovery (US FDA CVM, 2006).

Estimations of withdrawal period were completed for kidney and skeletal muscle tissues using FDA and CVMP guidelines (FDA, 2006; CVMP, 1995) with the following criteria.

- For a given time point to be included in the analysis, at least three animals had to have returned residues above the method limit of quantification (US FDA CVM, 2006)
- Nominal values that were below the limit of quantification, but above the limit of detection, were used if there were at least three points at the withdrawal period above the limit of quantification
- Values falling below the limit of detection were included at ½ the method limit of detection and were included in withdrawal period calculations (CVMP, 1995)

For kidney calculations, data obtained from withdrawal days 10, 15, 20, and 25 were used in the analysis. Data from day 32 were not employed even though there were 3 animals with penicillin residues above the LOQ because the 32 day data did not continue the linear trend with respect to days 20 and 25. For skeletal muscle, only one animal out of 18 had residues above the LOQ at the 15-d withdrawal period; 3 animals had residues above the LOD, but below the LOQ. Because a minimum of 3 days of data are required to determine the terminal, linear elimination period, the 15-d withdrawal period data were used for estimation of a withdrawal period, even though these data did not strictly comply with FDA guidelines. Excel 7.0 was used in conjunction with the tables of Owen (1962) to calculate the critical values or the non-central t-distribution used in FDA calculations. Withdrawal periods were estimated using the FSIS action level for penicillin G of 25 ppb (FSIS, 2013).

**Results**

Injection pattern had no discernible effect on residue depletion, so data were pooled across treatment. The depletion of ln-transformed penicillin G residues from kidneys of heavy sows are presented in Figure 1 as geometric means. Penicillin residues were measured in kidneys of all animals 5 days after the final treatment, but by 10 days 6 of 18 animals had residues below the assay LOD (1.8 ng/g). By 20 days of withdrawal, 5 of 18 animals had residues above the assay LOQ (6.1 ng/g) with 3 of these animals having kidney residues above 100 ng/g and one animal with penicillin residues greater than 300 ng/g. Penicillin residues (22.7 and 17.0 ng/g) were present in kidneys of two hogs after a 39-d withdrawal period. The estimated withdrawal period to ensure penicillin G depletion to a 25 ng/g action level in 99% of animals with 95% confidence was 52 days.

In contrast to kidney tissues, Penicillin G residues depleted quickly from skeletal muscle (Figure 2). Residues in skeletal muscle at 5 days of withdrawal averaged only 23.5 ± 10.5 ng/g and depleted rapidly thereafter. By the 15th day of withdrawal, only 1 sow had skeletal muscle residues greater than the assay LOQ (2.4 ng/g) with 4 other sows having residues greater than the method LOD (0.7 ng/g). Thus, the estimated withdrawal period for skeletal muscle was calculated to be 13 days (Figure 2).
Discussion
A withdrawal period was estimated for kidney tissues using the log-linear approach promulgated by the US-FDA CVM (2006) with modifications suggested by the CVMP (1995). In making a withdrawal period estimation, the essential assumptions of equal variance and normal distributions (Shapiro-Wilk) of data were violated (P < 0.001), thus the estimated withdrawal period presented here was admittedly calculated with data that did not conform to statistical ideals. As discussed by Concordet and Toutain (1997a) and documented by Sanquer et al. (2006) these assumptions are, at best, difficult, and are sometimes impossible to meet. Non parametric approaches to withdrawal period calculations proffered by Sanquer et al (2006) and Concordet and Toutain (1997b) were not attempted on this data set.

While the time required for kidney residues to deplete to FSIS action levels (52 d) far exceeded the FARAD estimated withdrawal time of 15 days for extra-label penicillin use, 15 d was more than sufficient for penicillin residues to deplete from skeletal muscle.

Conclusion
The use of penicillin G procaine as an economical and effective therapeutic drug for use in heavy sows will likely require the guarantee that kidneys from treated animals be included as offal not fit for human consumption.

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References

Figure 2. Penicillin G depletion from skeletal muscle of heavy sows. Geometric means of ln-transformed residues are shown. Hatched lines are the 25 ng/g action level, the calculated action level limit, and corresponding time (d) to for penicillin levels in 99% of animals to fall to the action limit with 95% confidence.